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(54) Title: PORCINE CIRCOVIRUS RECOMBINANT POXVIRUS VACCINE

(57) Abstract: What is described is a recombinant poxvirus, such as avipox virus, containing foreign DNA from porcine circovirus 2. What are also described are immunological compositions containing the recombinant poxvirus for inducing an immunological response in a host animal to which the immunological composition is administered. Also described are methods of treating or preventing disease caused by porcine circovirus 2 by administering the immunological compositions of the invention to an animal in need of treatment or susceptible to infection by porcine circovirus 2.

WO 00/77216 A2

**TITLE OF THE INVENTION**

*Porcine Circovirus Recombinant Poxvirus Vaccine*

**CROSS REFERENCE TO RELATED APPLICATIONS**

This application claims priority from U.S. application Serial No. 60/138,478,  
5 filed June 10, 1999 and from the U.S. utility application filed May 31, 2000.

Reference is made to WO-A-99 18214, 1998, French applications Nos. 97/12382,  
98/00873, 98/03707, filed October 3, 1997, January 22, 1998, and March 20, 1998,  
and WO99/29717. Each of the aforementioned U.S., PCT and French applications,  
and each document cited in the text and the record or prosecution of each of the  
10 aforementioned U.S., PCT and French applications ("application cited documents")  
and each document referenced or cited in each of the application cited documents, is  
hereby incorporated herein by reference; and, technology in each of the  
aforementioned U.S., PCT and French applications, and each document cited in the  
text and the record or prosecution of each of the aforementioned U.S., PCT and  
15 French applications can be used in the practice of this invention.

Several publications are referenced in this application. Full citation to these  
documents is found at the end of the specification preceding the claims, and/or where  
the document is cited. These documents pertain to the field of this invention; and,  
each of the documents cited or referenced in this application ("herein cited  
20 documents") and each document cited or referenced in herein cited documents are  
hereby incorporated herein by reference.

**FIELD OF THE INVENTION**

The present invention relates to vectors, such as recombinant vectors; for  
instance, recombinant viruses, such as poxviruses, e.g., modified poxviruses and to  
25 methods of making and using the same. In some embodiments, the invention relates to  
recombinant avipox viruses, such as canarypox viruses, e.g., ALVAC. The invention  
further relates to such vectors, e.g., poxviruses, that express gene products, e.g.,  
antigen(s), ORF(s), and/or epitope(s) of interest therefrom, of porcine circovirus 2  
(PCV2); to immunological compositions or vaccines. The invention yet further  
30 relates to such vectors, e.g., poxviruses, that induce an immune response directed to or  
against PCV2 gene products and/or PCV2; and, to advantageously, such compositions  
that are immunological, immunogenic or vaccine compositions and/or confer  
protective immunity against infection by PCV2. The invention yet further relates to

the uses of and methods for making and using such vectors and compositions, as well as intermediates thereof, and said intermediates. And, the invention relates to the products therefrom, e.g., from the uses and methods involving the inventive recombinant or poxvirus, such as antibodies from expression.

## 5 **BACKGROUND OF THE INVENTION**

Postweaning multisystemic wasting syndrome (PMWS) is a recently recognized disease of young pigs. PMWS is characterized clinically by progressive weight loss and other symptoms such as tachypnea, dyspnea and jaundice. Pathologically, lymphocytic and granulomatous infiltrates, lymphadenopathy, and, 10 more rarely, lymphocytic and granulomatous hepatitis and nephritis have been observed (Clark, 1997; Harding, 1997).

This disease has been described in different European countries as well as in North America. Treatment and prevention of this disease are not currently available.

Several lines of evidence point to porcine circovirus as the etiologic agent of 15 PMWS (Ellis et al., 1998). Circoviruses have been recovered from pigs with PMWS, and antibodies to porcine circovirus have been demonstrated in pigs with the disease.

Circoviruses are single stranded circular DNA viruses found in a range of animal and plant species. Porcine circovirus was originally isolated as a contaminant from a continuous pig kidney cell line. The cell culture isolate has been designated 20 PK-15 (Meehan et al., 1997). More recently, porcine circovirus obtained from pigs with PMWS has been compared to PK-15. Such viruses differ substantially from PK-15 at the nucleotide and protein sequence level, and have been designated PCV2 (Meehan et al., 1998; Hamel et al., 1998).

As many as thirteen open reading frames (ORFs) have been identified in the 25 PCV2 genome (COL1 to COL13 in the French patent application 98 03707). Four of these ORFs share substantial homology with analogous ORFs within the genome of PK-15. ORF1 (Meehan et al., 1998; corresponding to COL4 in the French patent application 98 03707), comprising nt 398-1342 (GenBank accession number AF055392), has the potential to encode a protein with a predicted molecular weight of 30 37.7 kD. ORF2 (Meehan et al., 1998; corresponding to COL13 in the French patent application 98 03707), comprising nt 1381-1768 joined to 1-314 (GenBank accession number AF055392), may encode a protein with a predicted molecular weight of 27.8 kD. ORF3 (Meehan et al., 1998; corresponding to COL7 in the French patent

application 98 03707), comprising nt 1018-704 (GenBank accession number AF055392), may encode a protein with a predicted molecular weight of 11.9 kD. ORF4 (Meehan et al., 1998; corresponding to COL10 in the French patent application 98 03707), comprising nt 912-733 (GenBank accession number AF055392), may  
5 encode a protein with a predicted molecular weight of 6.5 kD.

ORF1 of PCV2 is highly homologous (86% identity) to the ORF1 of the PK-15 isolate (Meehan et al., 1998). The ORF1 protein of PK-15 has been partially characterized (Meehan et al., 1997 ; Mankertz et al., 1998a). It is known to be essential for virus replication, and is probably involved in the viral DNA replication.

10 Protein sequence identity between the respective ORF2s was lower (66% identity) than that of the ORF1s but each of the ORF2s shared a highly conserved basic N-terminal region, similar to that observed in the N-terminal region of the major structural protein of the avian circovirus chicken anemia virus (CAV) (Meehan et al., 1998). Recently, Mankertz et al. (1998b) has suggested that the ORF2 of the PK-15  
15 isolate (designated ORF 1 in Mankertz et al., 1998b) codes for a capsid protein.

Greater differences were observed between the respective ORF3s and ORF4s of the PK-15 isolate and PCV2. In each case, there was a deletion of the C-terminal region of PCV2 ORF4 and ORF3 compared to the corresponding ORFs present in the genome of the PK-15 isolate. The highest protein sequence homology was observed at  
20 the N-terminal regions of both ORF3 and ORF4 (Meehan et al., 1998).

The transcription analysis of the genome of PCV2 has not been published yet. Recent data obtained with the PK-15 isolate indicated that the ORF2 transcript is spliced (Mankertz et al., 1998b).

Vaccinia virus has been used successfully to immunize against smallpox,  
25 culminating in the worldwide eradication of smallpox in 1980. With the eradication of smallpox, a new role for poxviruses became important, that of a genetically engineered vector for the expression of foreign genes (Panicali and Paoletti, 1982; Paoletti et al., 1984). Genes encoding heterologous antigens have been expressed in vaccinia, often resulting in protective immunity against challenge by the  
30 corresponding pathogen (reviewed in Tartaglia et al., 1990). A highly attenuated strain of vaccines, designated MVA, has also been used as a vector for poxvirus-based vaccines. Use of MVA is described in U.S. Patent No. 5,185,146.

Two additional vaccine vector systems involve the use of naturally host-restricted poxviruses, avipox viruses. Both fowlpoxvirus (FPV; Taylor et al. 1988a, b) and canarypoxvirus (CPV; Taylor et al., 1991 & 1992) have been engineered to express foreign gene products. Fowlpox virus (FPV) is the prototypic virus of the Avipox genus of the Poxvirus family. The virus causes an economically important disease of poultry which has been well controlled since the 1920's by the use of live attenuated vaccines. Replication of the avipox viruses is limited to avian species (Matthews, 1982) and there are no reports in the literature of avipoxvirus causing a productive infection in any non-avian species including man. This host restriction provides an inherent safety barrier to transmission of the virus to other species and makes use of avipoxvirus based vaccine vectors in veterinary and human applications an attractive proposition.

FPV has been used advantageously as a vector expressing antigens from poultry pathogens. The hemagglutinin protein of a virulent avian influenza virus was expressed in an FPV recombinant (Taylor et al., 1988c). After inoculation of the recombinant into chickens and turkeys, an immune response was induced which was protective against either a homologous or a heterologous virulent influenza virus challenge (Taylor et al., 1988c). FPV recombinants expressing the surface glycoproteins of Newcastle Disease Virus have also been developed (Taylor et al., 1990 ; Edbauer et al., 1990).

Other attenuated poxvirus vectors have been prepared by genetic modifications of wild type strains of virus. The NYVAC vector, derived by deletion of specific virulence and host-range genes from the Copenhagen strain of vaccinia (Tartaglia et al., 1992) has proven useful as a recombinant vector in eliciting a protective immune response against an expressed foreign antigen.

Another engineered poxvirus vector is ALVAC, derived from canarypox virus. ALVAC does not productively replicate in non-avian hosts, a characteristic thought to improve its safety profile (Taylor et al., 1991 & 1992). Both ALVAC and NYVAC are BSL-1 vectors.

One approach to the development of a subunit PCV2 vaccine is the use of live viral vectors to express relevant PCV2 ORFs. Recombinant poxviruses can be constructed in two steps known in the art and analogous to the methods for creating synthetic recombinants of poxviruses such as the vaccinia virus and avipox virus

described in U.S. Patent Nos. 4,769,330; 4,722,848; 4,603,112; 5,110,587; 5,174,993; 5,494,807; and 5,505,941, the disclosures of which are incorporated herein by reference. It can thus be appreciated that provision of a PCV2 recombinant poxvirus, and of compositions and products therefrom particularly ALVAC based PCV2 recombinants and compositions and products therefrom, especially such recombinants containing ORFs 1 and/or 2 of PCV2, and compositions and products therefrom would be a highly desirable advance over the current state of technology.

### **OBJECTS AND SUMMARY OF THE INVENTION**

It is therefore an object of this invention to provide compositions and methods for treatment and prophylaxis of infection with PCV2. It is also an object to provide a means to treat or prevent PMWS.

In one aspect, the present invention relates to an antigenic, immunological, immunogenic, or vaccine composition or a therapeutic composition for inducing an antigenic, immunogenic or immunological response in a host animal inoculated with the composition. The composition advantageously includes a carrier or diluent and a recombinant virus, such as a recombinant poxvirus. The recombinant virus or poxvirus contains and expresses an exogenous nucleic acid molecule encoding an ORF, antigen, immunogen, or epitope of interest from PCV2, or a protein that elicits an immunological response against PCV2 or conditions caused by PCV2, such as PMWS. For instance, the recombinant virus can be a modified recombinant virus or poxvirus; for example, such a virus or poxvirus that has inactivated therein virus-encoded genetic functions, e.g., nonessential virus-encoded genetic functions, so that the recombinant virus has attenuated virulence and enhanced safety. And, the invention further provides the viruses used in the composition, as well as methods for making and uses of the composition and virus.

The virus used in the composition according to the present invention is advantageously a poxvirus, particularly a vaccinia virus or an avipox virus, such as fowlpox virus or canarypox virus and more advantageously, ALVAC. The modified recombinant virus can include, e.g., within a non-essential region of the virus genome, a heterologous DNA sequence which encodes an antigenic protein, e.g., derived from PCV2 ORFs, e.g., PCV2 ORF 1 and/or 2.

In yet another aspect, the present invention relates to an immunogenic composition containing a modified recombinant virus having inactivated nonessential

virus-encoded genetic functions so that the recombinant virus has attenuated virulence and enhanced safety. The modified recombinant virus includes, e.g., within a non-essential region of the virus genome, a heterologous DNA sequence which encodes an antigenic protein (e.g., derived from PCV2 ORFs, especially ORFS 1 and/or 2)

5 wherein the composition, when administered to a host, is capable of inducing an immunological response specific to the antigen.

In a still further aspect, the present invention relates to a modified recombinant virus having nonessential virus-encoded genetic functions inactivated therein so that the virus has attenuated virulence, and wherein the modified recombinant virus further  
10 contains DNA from a heterologous source, e.g., in a nonessential region of the virus genome. The DNA can code for PCV2 genes such as any or all of PCV2 ORF1, ORF2, ORF3, or ORF4 (Meehan et al., 1998), or epitope(s) of interest therefrom. The genetic functions can be inactivated by deleting an open reading frame encoding a virulence factor or by utilizing naturally host-restricted viruses. The virus used  
15 according to the present invention is advantageously a poxvirus, e.g., a vaccinia virus or an avipox virus, such as fowlpox virus or canarypox virus.

Advantageously, the open reading frame that is deleted from the poxvirus or virus genome is selected from the group consisting of J2R, B13R + B14R, A26L, A56R, C7L - K1L, and I4L (by the terminology reported in Goebel et al., 1990); and,  
20 the combination thereof. In this respect, the open reading frame comprises a thymidine kinase gene, a hemorrhagic region, an A type inclusion body region, a hemagglutinin gene, a host range gene region or a large subunit, ribonucleotide reductase; or, the combination thereof.

A suitable modified Copenhagen strain of vaccinia virus is identified as  
25 NYVAC (Tartaglia et al., 1992), or a vaccinia virus from which has been deleted J2R, B13R+B14R, A26L, A56R, C7L-K11 and I4L or a thymidine kinase gene, a hemorrhagic region, an A type inclusion body region, a hemagglutinin gene, a host range region, and a large subunit, ribonucleotide reductase (See also U.S. Patent No. 5,364,773, 5,494,807, and 5,762,938, with respect to NYVAC and vectors having  
30 additional deletions or inactivations from those of NYVAC that are also useful in the practice of this invention).

Preferably, the poxvirus vector is an ALVAC or, a canarypox virus which was attenuated, for instance, through more than 200 serial passages on chick embryo

fibroblasts (Rentschler vaccine strain), a master seed therefrom was subjected to four successive plague purifications under agar from which a plague clone was amplified through five additional passages. (See also U.S. Patent Nos. 5,756,103 and 5,766,599 with respect to ALVAC and TROVAC (an attenuated fowlpox virus useful in the practice of this invention); and U.S. Patents Nos. 6,004,777, 5,990,091, 5,770,212, 6,033,904, 5,869,312, 5,382,425, and WO 95/30018, with respect to vectors that also can be used in the practice of this invention, such as vectors having enhanced expression, vectors having functions deleted therefrom and vectors useful with respect to porcine hosts (for instance, vectors useful with porcine hosts can include a poxvirus, including a vaccinia virus, an avipox virus, a canarypox virus, and a swinepox virus), as well as with respect to terms used and teachings herein such as "immunogenic composition", "immunological composition", "vaccine", and "epitope of interest", and dosages, routes of administration, formulations, adjuvants, and uses for recombinant viruses and expression products therefrom).

The invention in yet a further aspect relates to the product of expression of the inventive recombinant poxvirus and uses therefor, such as to form antigenic, immunological or vaccine compositions for treatment, prevention, diagnosis or testing; and, to DNA from the recombinant poxvirus which is useful in constructing DNA probes and PCR primers.

These and other embodiments are disclosed or are obvious from and encompassed by the following detailed description.

#### **BRIEF DESCRIPTION OF THE DRAWINGS**

A better understanding of the present invention will be had by referring to the accompanying drawings, incorporated herein by reference, in which:

- FIG. 1 (SEQ ID NO:1) shows the nucleotide sequence of a 3.7 kilobase pair fragment of ALVAC DNA containing the C6 open reading frame.
- FIG. 2 shows the map of pJP102 donor plasmid.
- FIG. 3 (SEQ ID NO:8) shows the nucleotide sequence of the 2.5 kilobase pair fragment from pJP102 donor plasmid from the *KpnI* (position 653) to the *SacI* (position 3166) restriction sites.
- FIG. 4 shows the map of pJP105 donor plasmid.
- FIG. 5 shows the map of pJP107 donor plasmid.



- FIG. 6 (SEQ ID NO:11) shows the nucleotide sequence of the 3.6 kilobase pair fragment from pJP107 donor plasmid from the *KpnI* (position 653) to the *SacI* (position 4255) restriction sites.

#### **DETAILED DESCRIPTION**

5 In one aspect, the present invention relates to a recombinant virus, such as a recombinant poxvirus, containing therein a DNA sequence from PCV2, e.g., in a non-essential region of the poxvirus genome. The poxvirus is advantageously an avipox virus, such as fowlpox virus, especially an attenuated fowlpox virus, or a canarypox virus, especially an attenuated canarypox virus, such as ALVAC.

10 According to the present invention, the recombinant poxvirus expresses gene products of the foreign PCV2 gene. Specific ORFs of PCV2 are inserted into the poxvirus vector, and the resulting recombinant poxvirus is used to infect an animal. Expression in the animal of PCV2 gene products results in an immune response in the animal to PCV2. Thus, the recombinant poxvirus of the present invention may be  
15 used in an immunological composition or vaccine to provide a means to induce an immune response which may, but need not be, protective.

The administration procedure for recombinant poxvirus-PCV2 or expression product thereof, compositions of the invention such as immunological, antigenic or vaccine compositions or therapeutic compositions, can be via a parenteral route  
20 (intradermal, intramuscular or subcutaneous). Such an administration enables a systemic immune response, or humoral or cell-mediated responses.

More generally, the inventive poxvirus- PCV2 recombinants, antigenic, immunological or vaccine poxvirus- PCV2 compositions or therapeutic compositions can be prepared in accordance with standard techniques well known to those skilled in  
25 the pharmaceutical or veterinary art. Such compositions can be administered in dosages and by techniques well known to those skilled in the medical or veterinary arts taking into consideration such factors as the age, sex, weight, species and condition of the particular patient, and the route of administration. The compositions can be administered alone, or can be co-administered or sequentially administered  
30 with compositions, e.g., with "other" immunological, antigenic or vaccine or therapeutic compositions thereby providing multivalent or "cocktail" or combination compositions of the invention and methods employing them. Again, the ingredients and manner (sequential or co-administration) of administration, as well as dosages can

be determined taking into consideration such factors as the age, sex, weight, species and condition of the particular patient, and, the route of administration. In this regard, reference is made to U.S. Patent No. 5,843,456, incorporated herein by reference, and directed to rabies compositions and combination compositions and uses thereof.

5           Examples of compositions of the invention include liquid preparations for orifice, e.g., oral, nasal, anal, vaginal, peroral, intragastric, etc., administration such as suspensions, syrups or elixirs; and, preparations for parenteral, subcutaneous, intradermal, intramuscular or intravenous administration (e.g., injectable administration) such as sterile suspensions or emulsions. In such compositions the  
10 recombinant poxvirus or antigens may be in admixture with a suitable carrier, diluent, or excipient such as sterile water, physiological saline, glucose or the like. The compositions can also be lyophilized. The compositions can contain auxiliary substances such as wetting or emulsifying agents, pH buffering agents, adjuvants, gelling or viscosity enhancing additives, preservatives, flavoring agents, colors, and  
15 the like, depending upon the route of administration and the preparation desired. Standard texts, such as "REMINGTON'S PHARMACEUTICAL SCIENCE", 17th edition, 1985, incorporated herein by reference, may be consulted to prepare suitable preparations, without undue experimentation. Suitable dosages can also be based upon the Examples below.

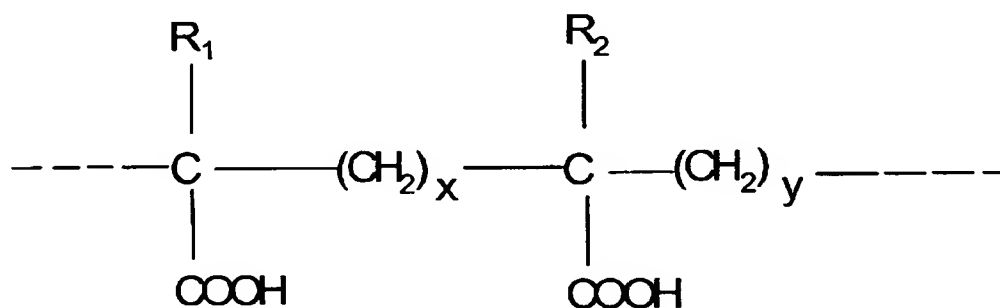
20           The compositions can contain at least one adjuvant compound chosen from the polymers of acrylic or methacrylic acid and the copolymers of maleic anhydride and alkenyl derivative.

          The preferred adjuvant compounds are the polymers of acrylic or methacrylic acid which are cross-linked, especially with polyalkenyl ethers of sugars or  
25 polyalcohols. These compounds are known by the term carbomer (Pharmeuropa Vol. 8, No. 2, June 1996). Persons skilled in the art can also refer to U.S. Patent No. 2,909,462 (incorporated herein by reference) which describes such acrylic polymers cross-linked with a polyhydroxylated compound having at least 3 hydroxyl groups, preferably not more than 8, the hydrogen atoms of at least three hydroxyls being  
30 replaced by unsaturated aliphatic radicals having at least 2 carbon atoms. The preferred radicals are those containing from 2 to 4 carbon atoms, e.g. vinyls, allyls and other ethylenically unsaturated groups. The unsaturated radicals may themselves contain other substituents, such as methyl. The products sold under the name

Carbopol® (BF Goodrich, Ohio, USA) are particularly appropriate. They are cross-linked with an allyl sucrose or with allyl pentaerythritol. Among them, there may be mentioned Carbopol® 974P, 934P and 971P.

Among the copolymers of maleic anhydride and alkenyl derivative, the copolymers EMA® (Monsanto) which are copolymers of maleic anhydride and ethylene, linear or cross-linked, for example cross-linked with divinyl ether, are preferred. Reference may be made to J. Fields et al., Nature, 186 : 778-780, 4 June 1960, incorporated herein by reference.

From the point of view of their structure, the polymers of acrylic or methacrylic acid and the copolymers EMA® are preferably formed of basic units of the following formula :



in which :

- R<sub>1</sub> and R<sub>2</sub>, which are identical or different, represent H or CH<sub>3</sub>,
- x = 0 or 1, preferably x = 1
- y = 1 or 2, with x + y = 2

For the copolymers EMA®, x = 0 and y = 2. For the carbomers, x = y = 1.

The dissolution of these polymers in water leads to an acid solution which will be neutralized, preferably to physiological pH, in order to give the adjuvant solution into which the vaccine itself will be incorporated. The carboxyl groups of the polymer are then partly in COO<sup>-</sup> form.

Preferably, a solution of adjuvant according to the invention, especially of carbomer, is prepared in distilled water, preferably in the presence of sodium chloride, the solution obtained being at acidic pH. This stock solution is diluted by adding it to the desired quantity (for obtaining the desired final concentration), or a substantial part thereof, of water charged with NaCl, preferably physiological saline (NaCl 9 g/l) all at once in several portions with concomitant or subsequent neutralization (pH 7.3 to 7.4), preferably with NaOH. This solution at physiological pH will be used as it is for mixing with the vaccine, which may be especially stored in freeze-dried, liquid or frozen form.

The polymer concentration in the final vaccine composition will be 0.01% to 2% w/v, more particularly 0.06 to 1% w/v, preferably 0.1 to 0.6% w/v. The immunological compositions according to the invention may be associated to at least one live attenuated, inactivated, or sub-unit vaccine, or recombinant vaccine (e.g. poxvirus as vector or DNA plasmid) expressing at least one immunogen from another pig pathogen.

The invention encompasses vectors encoding and expressing equivalent nucleotide sequences, that is to say the sequences which change neither the functionality or the strain specificity (say strain of type 1 and strain of type 2) of the gene considered or those of the polypeptides encoded by this gene. The sequences differing through the degeneracy of the code are, of course, included.

The PCV-2 sequences used in the examples are derived from Meehan *et al.* (Strain Imp.1010 ; ORF1 nucleotides 398-1342; ORF2 nucleotides 1381-314; and correspond respectively to ORF4 and ORF13 in U.S. application Serial No. 09/161,092 of 25 September 1998 and to COL4 and COL13 in WO-A-9918214).

Other PCV-2 strains and their sequences have been published in WO-A-9918214 and are called Imp1008, Imp999, Imp1011-48285 and Imp1011-48121, as well as in A.L. Hamel *et al.* J. Virol. June 1998, vol 72, 6: 5262-5267 (GenBank AF027217) and in I. Morozov *et al.* J. Clinical Microb. Sept. 1998 vol. 36, 9: 2535-2541, as well as GenBank AF086834, AF086835 and AF086836, and give access to equivalent ORF sequences. These sequences, or ORFs therefrom, or regions thereof encoding an antigen or epitope of interest can also be used in the practice of this invention.

The invention also encompasses the equivalent sequences to those used herein and in documents cited herein; for instance, sequences that are capable of hybridizing

to the nucleotide sequence under high stringency conditions (see, e.g., Sambrook et al. (1989). Among the equivalent sequences, there may also be mentioned the gene fragments conserving the immunogenicity of the complete sequence, e.g., an epitope of interest.

5           The homology of the whole genome between PCV types 1 and 2 is about 75%. For ORF1, it is about 86%, and for ORF2, about 66%. On the contrary, homologies between genomes and between ORFs within type 2 are generally above 95%.

          Also, equivalent sequences useful in the practice of this present invention, for ORF1, are those sequences having an homology equal or greater than 88%,  
10   advantageously 90% or greater, preferably 92% or 95% or greater with ORF1 of strain Imp1010, and for ORF2, are those sequences having an homology equal or greater than 80%, advantageously 85% or greater, preferably 90% or 95% or greater with ORF2 of strain Imp1010.

          ORF1 and ORF2 according to Meehan 1998 has the potential to encode  
15   proteins with predicted molecular weights of 37.7 kD and 27.8 kD respectively. ORF3 and ORF4 (according to Meehan et al. 1998, correspond to ORF7 and ORF10 respectively in WO-A-9918214) has the potential to encode proteins with predicted molecular weights of 11.9 and 6.5 kD respectively. The sequence of these ORFs is also available in Genbank AF 055392. They can also be incorporated in plasmids and  
20   be used in accordance with the invention alone or in combination, e.g. with ORF1 and/or ORF2.

          The other ORFs 1-3 and 5, 6, 8-9, 11-12 disclosed in U.S. application Serial No. 09/161,092 of 25 September 1998 (COLs 1-3 and 5, 6, 8-9, 11-12 in WO-A-9918214), or region(s) thereof encoding an antigen or epitope of interest, may be used  
25   in the practice of this invention, e.g., alone or in combination or otherwise with each other or with the ORFs 1 and 2 or region(s) thereof encoding antigen(s) or epitope(s).

          This invention also encompasses the use of equivalent sequences; for instance, from ORFs of various PCV-2 strains cited herein. For homology, one can determine that there are equivalent sequences which come from a PCV strain having an ORF2  
30   and/or an ORF1 which have an homology as defined above with the corresponding ORF of strain 1010.

          For ORF3 according to Meehan, an equivalent sequence has homology thereto that is advantageously, for instance, equal or greater than 80%, for example 85% or

greater, preferably 90% or 95% or greater with ORF3 of strain Imp1010. For ORF4 according to Meehan 1998, advantageously an equivalent sequence has homology that is equal or greater than 86%, advantageously 90% or greater, preferably than 95% or greater with ORF4 of strain Imp1010.

5 From the genomic nucleotide sequence, e.g. those disclosed in WO-A-99 18214, it is routine art to determine the ORFs using a standard software, such as MacVector®. Also, alignment of genomes with that of strain 1010 and comparison with strain 1010 ORFs allows the one skilled in the art to readily determine the ORFs of the genome of another strain (e.g. other strains disclosed in WO-A-99 18214 or in  
10 other herein cited documents).

Using software or making sequence alignment is not undue experimentation and provides direct access to equivalent ORFs or nucleic acid molecules.

Nucleotide sequence homology can be determined using the "Align" program of Myers and Miller, ("Optimal Alignments in Linear Space", CABIOS 4, 11-17,  
15 1988, incorporated herein by reference) and available at NCBI. Alternatively or additionally, the term "homology" or "identity", for instance, with respect to a nucleotide or amino acid sequence, can indicate a quantitative measure of homology between two sequences. The percent sequence homology can be calculated as  $(N_{ref} - N_{dif}) * 100 / N_{ref}$ , wherein  $N_{dif}$  is the total number of non-identical residues in the two  
20 sequences when aligned and wherein  $N_{ref}$  is the number of residues in one of the sequences. Hence, the DNA sequence AGTCAGTC will have a sequence similarity of 75% with the sequence AATCAATC ( $N_{ref} = 8$ ;  $N_{dif} = 2$ ).

Alternatively or additionally, "homology" or "identity" with respect to sequences can refer to the number of positions with identical nucleotides or amino  
25 acids divided by the number of nucleotides or amino acids in the shorter of the two sequences wherein alignment of the two sequences can be determined in accordance with the Wilbur and Lipman algorithm (Wilbur and Lipman, 1983 PNAS USA 80:726, incorporated herein by reference), for instance, using a window size of 20 nucleotides, a word length of 4 nucleotides, and a gap penalty of 4, and computer-  
30 assisted analysis and interpretation of the sequence data including alignment can be conveniently performed using commercially available programs (e.g., Intelligenetics™ Suite, Intelligenetics Inc. CA).. When RNA sequences are said to be similar, or

have a degree of sequence identity or homology with DNA sequences, thymidine (T) in the DNA sequence is considered equal to uracil (U) in the RNA sequence.

RNA sequences within the scope of the invention can be derived from DNA sequences, by thymidine (T) in the DNA sequence being considered equal to uracil (U) in RNA sequences.

Additionally or alternatively, amino acid sequence similarity or identity or homology can be determined using the BlastP program (Altschul *et al.*, Nucl. Acids Res. 25, 3389-3402, incorporated herein by reference) and available at NCBI. The following references (each incorporated herein by reference) provide algorithms for comparing the relative identity or homology of amino acid residues of two proteins, and additionally or alternatively with respect to the foregoing, the teachings in these references can be used for determining percent homology or identity: Needleman SB and Wunsch CD, "A general method applicable to the search for similarities in the amino acid sequences of two proteins," J. Mol. Biol. 48:444-453 (1970); Smith TF and Waterman MS, "Comparison of Bio-sequences," Advances in Applied Mathematics 2:482-489 (1981); Smith TF, Waterman MS and Sadler JR, "Statistical characterization of nucleic acid sequence functional domains," Nucleic Acids Res., 11:2205-2220 (1983); Feng DF and Dolittle RF, "Progressive sequence alignment as a prerequisite to correct phylogenetic trees," J. of Molec. Evol., 25:351-360 (1987); Higgins DG and Sharp PM, "Fast and sensitive multiple sequence alignment on a microcomputer," CABIOS, 5: 151-153 (1989); Thompson JD, Higgins DG and Gibson TJ, "ClusterW: improving the sensitivity of progressive multiple sequence alignment through sequence weighing, positions-specific gap penalties and weight matrix choice," Nucleic Acid Res., 22:4673-480 (1994); and, Devereux J, Haeberlie P and Smithies O, "A comprehensive set of sequence analysis program for the VAX," Nucl. Acids Res., 12: 387-395 (1984).

This invention not only allows for administration to adult pigs, but also to the young and to gestating females; in the latter case, this makes it possible, in particular, to confer passive immunity onto the newborns (maternal antibodies). Preferably, female pigs are inoculated prior to breeding; and/or prior to serving, and/or during gestation. Advantageously, at least one inoculation is done before serving and it is preferably followed by an inoculation to be performed during gestation, e.g., at about mid-gestation (at about 6-8 weeks of gestation) and/or at the end of gestation (at about

11-13 weeks of gestation). Thus, an advantageous regimen is an inoculation before mating and/o serving and a booster inoculation during gestation. Thereafter, there can be reinoculation before each serving and/or during gestation at about mid-gestation and/or at the end of gestation. Preferably, reinoculations are during gestation. Male  
5 pigs also can be inoculated, e.g., prior to mating.

Piglets, such as piglets from vaccinated females (e.g., inoculated as herein discussed), are inoculated within the first weeks of life, e.g., inoculation at one and/or two and/or three and/or four and/or five weeks of life. Preferably, piglets are first  
10 inoculated within the first week of life or within the third week of life (e.g., at the time of weaning). Advantageously, such piglets are then boosted two to four weeks later.

The present invention is additionally described by the following illustrative, non-limiting Examples.

### EXAMPLES

The invention in a preferred embodiment is directed to recombinant  
15 poxviruses containing therein a DNA sequence from PCV2 in a nonessential region of the poxvirus genome. The recombinant poxviruses express gene products of the foreign PCV2 gene. In particular, ORF2 and ORF1 genes encoding PCV2 proteins were isolated, characterized and inserted into ALVAC (canarypox vector) recombinants. The molecular biology techniques used are the ones described by  
20 Sambrook et al. (1989).

Cell Lines and Virus Strains. The strain of PCV2 designated Imp.1010-Stoon has been previously described (Meehan et al., 1998). It was isolated from mesenteric lymph node tissues from a diseased pig originating from Canada. Cloning of the PCV2 genome was described by Meehan et al. (1998). Plasmid pGem7Z-Imp1010-  
25 Stoon-EcoRI No. 14 contains the PCV2 genome as an *EcoRI* fragment inserted into the *EcoRI* site of plasmid pGem-7Z (Promega, Madison, WI). The complete nucleotide sequence of the Imp.1010-Stoon PCV2 strain has been determined by Meehan et al. (1998) and is available under the GenBank accession number AF055392.

30 The parental canarypox virus (Rentschler strain) is a vaccinal strain for canaries. The vaccine strain was obtained from a wild type isolate and attenuated through more than 200 serial passages on chick embryo fibroblasts. A master viral seed was subjected to four successive plaque purifications under agar and one plaque



clone was amplified through five additional passages after which the stock virus was used as the parental virus in *in vitro* recombination tests. The plaque purified canarypox isolate is designated ALVAC. ALVAC was deposited November 14, 1996 under the terms of the Budapest Treaty at the American Type Culture Collection,

5 ATCC accession number VR-2547.

The generation of poxvirus recombinants involves different steps: (1) construction of an insertion plasmid containing sequences ("arms") flanking the insertion locus within the poxvirus genome, and multiple cloning site (MCS) localized between the two flanking arms (c.g., see Example 1); (2) construction of donor  
10 plasmids consisting of an insertion plasmid into the MCS of which a foreign gene expression cassette has been inserted (c.g. see Examples 2 to 5); (3) *in vitro* recombination in cell culture between the arms of the donor plasmid and the genome of the parental poxvirus allowing the insertion of the foreign gene expression cassette into the appropriate locus of the poxvirus genome, and plaque purification of the  
15 recombinant virus (e.g. see Example 6).

PCV2 recombinant immunogens may be used in association with PCV1 immunogens, for immunization of animals against PMWS. In a least preferred approach, PCV1 immunogens may be used without PCV2 immunogens.

20 **Example 1** - **CONSTRUCTION OF CANARYPOX  
INSERTION PLASMID AT C6 LOCUS**

Figure 1 (SEQ ID NO:1) is the sequence of a 3.7 kb segment of canarypox DNA. Analysis of the sequence revealed an ORF designated C6L initiated at position 377 and terminated at position 2254. The following describes a C6 insertion plasmid constructed by deleting the C6 ORF and replacing it with a multiple cloning site  
25 (MCS) flanked by transcriptional and translational termination signals. A 380 bp PCR fragment was amplified from genomic canarypox DNA using oligonucleotide primers C6A1 (SEQ ID NO:2) and C6B1 (SEQ ID NO:3). A 1155 bp PCR fragment was amplified from genomic canarypox DNA using oligonucleotide primers C6C1 (SEQ ID NO:4) and C6D1 (SEQ ID NO:5). The 380 bp and 1155 bp fragments were fused  
30 together by adding them together as template and amplifying a 1613 bp PCR fragment using oligonucleotide primers C6A1 (SEQ ID NO:2) and C6D1 (SEQ ID NO:5). This fragment was digested with *SacI* and *KpnI*, and ligated into pBluescript SK+ (Stratagene, La Jolla, CA, USA) digested with *SacI/KpnI*. The resulting plasmid,

pC6L was confirmed by DNA sequence analysis. It consists of 370 bp of canarypox DNA upstream of C6 ("C6 left arm"), vaccinia early termination signal, translation stop codons in six reading frames, an MCS containing *SmaI*, *PstI*, *XhoI* and *EcoRI* sites, vaccinia early termination signal, translation stop codons in six reading frames and 1156 bp of downstream canary pox sequence ("C6 right arm").

Plasmid pJP099 was derived from pC6L by ligating a cassette containing the vaccinia H6 promoter (described in Taylor et al. (1988c), Guo et al. (1989), and Perkus et al. (1989)) coupled to a foreign gene into the *SmaI/EcoRI* sites of pC6L. This plasmid pJP099 contains a unique *EcoRV* site and a unique *NruI* site located at the 3' end of the H6 promoter, and a unique *SalI* site located between the STOP codon of the foreign gene and the C6 left arm. The ~4.5 kb *EcoRV/SalI* or *NruI/SalI* fragment from pJP099 contains therefore the plasmid sequence (pBluescript SK+ ; Stratagene, La Jolla, CA, USA), the 2 C6 arms and the 5' end of the H6 promoter until the *EcoRV* or *NruI* site.

#### Sequences of the primers:

Primer C6A1 (SEQ ID NO:2)

ATCATCGAGCTCGCGGCCGCCTATCAAAAGTCTTAATGAGTT

Primer C6B1 (SEQ ID NO:3)

GAATTCCTCGAGCTGCAGCCCGGGTTTTATAGCTAATTAGTCATTTTTTC

GTAAGTAAGTATTTTTATTTAA

Primer C6C1 (SEQ ID NO:4)

CCCGGGCTGCAGCTCGAGGAATTCTTTTTATTGATTAAGTCAAATGAG

TATATATAATTGAAAAAGTAA

Primer C6D1 (SEQ ID NO:5)

GATGATGGTACCTTCATAAATACAAGTTTGATTAAACTTAAGTTG

#### Example 2 - CONSTRUCTION OF ALVAC DONOR PLASMID FOR PCV2 ORF2

Plasmid pGem7Z-Imp1010-Stoon-EcoRI No. 14, containing the PCV2 genome as an *EcoRI* fragment in plasmid pGem-7Z, was digested with *EcoRI*, and a 1768bp fragment was isolated and ligated.

In order to insert PCV2 ORF 2 into an appropriate ALVAC insertion vector: Primers JP760 (SEQ ID NO:6) and JP773 (SEQ ID NO:7) were used to amplify PCV2 ORF 2 from the 1768bp ligated *EcoRI* fragment (see above) resulting in PCR

J1304. Primer JP760 (SEQ ID NO:6) contains the 3' end of the H6 promoter from *EcoRV* and the 5' end of PCV2 ORF 2. Primer JP773 (SEQ ID NO:7) contains the 3' end of PCV2 ORF 2 followed by a *SaII* site. The product of PCR J1304 was then digested with *EcoRV/SaII* and cloned as a ~750 bp fragment into a ~4.5 kb

*EcoRV/SaII* fragment from pJP099 (see above in Example 1). The resulting plasmid was confirmed by sequence analysis and designated pJP102 (see the map of pJP102 in Figure 2 and the sequence (SEQ ID NO:8) in Figure 3). The sequence of ORF 2 matches sequence available in GenBank, Accession Number AF055392. The donor plasmid pJP102 (linearized with *NotI*) was used in an *in vitro* recombination (IVR) test to generate ALVAC recombinant vCP1614 (see Example 6).

Sequence of the primers:

JP760 (SEQ ID NO:6)

CAT-CAT-CAT-GAT-ATC-CGT-TAA-GTT-TGT-ATC-GTA-ATG-ACG-TAT-CCA-AGG-AGG-CG

JP773 (SEQ ID NO:7)

TAC-TAC-TAC-GTC-GAC-TTA-GGG-TTT-AAG-TGG-GGG-GTC

**Example 3 - CONSTRUCTION OF AN ALVAC DONOR PLASMID FOR PCV2 ORF2 AND ORF1**

PCV2 ORF 1 was amplified by PCR using primers JP774 (SEQ ID NO:9) and JP775 (SEQ ID NO:10) on plasmid pGem7Z-Imp1010-Stoon-EcoRI No. 14 resulting in PCR J1311. Primer JP774 (SEQ ID NO:9) contains the 3' end of the H6 promoter from *NruI* and the 5' end of PCV2 ORF1. Primer JP775 (SEQ ID NO:10) contains the 3' end of PCV2 ORF1 followed by a *SaII* site. The product of PCR J1311 (~1 Kb) was cloned into pCR2.1 (Invitrogen, Carlsbad, CA). The resulting plasmid was confirmed by sequence analysis and designated pJP104. The sequence of ORF1 matches sequence available in GenBank, Accession Number AF055392. A ~970 bp *NruI/SaII* fragment was isolated from pJP104 and cloned into a ~4.5 kb *NruI/SaII* fragment from pJP099 (see Example 1), resulting in a plasmid which was confirmed by restriction analysis and designated pJP105 (see Figure 4). The donor plasmid pJP105 could be used in an *in vitro* recombination test (described in Example 6) to generate ALVAC recombinant expressing the PCV2 ORF1.

A ~838bp *BamHI/SaII* from pJP102 (see Example 2) was blunted using the Klenow fragment of DNA polymerase, and was cloned into the Klenow-blunted

*EcoRI* site of pJP105. Clones were checked for orientation of insert by restriction analysis and a head-to-head orientation was chosen. This plasmid was confirmed by sequence analysis and designated pJP107 (see the map of pJP107 in Figure 5 and the sequence (SEQ ID NO:11) in Figure 6). The donor plasmid pJP107 (linearized with *NotI*) was used in an *in vitro* recombination (IVR) test to generate the ALVAC recombinant vCP1615 (see Example 6).

Sequence of the primers:

JP774 (SEQ ID NO:9)

CAT-CAT-CAT-TCG-CGA-TAT-CCG-TTA-AGT-TTG-TAT-CGT-AAT-GCC-

CAG-CAA-GAA-GAA-TGG

JP775 (SEQ ID NO:10)

TAC-TAC-TAC-GTC-GAC-TCA-GTA-ATT-TAT-TTC-ATA-TGG

**Example 4 - CONSTRUCTION OF ALVAC  
DONOR PLASMID FOR PCV1 ORF2**

Plasmid pPCV1 (B. Meehan *et al.* J. Gen. Virol. 1997. 78. 221-227), containing the PCV1 genome as a *PstI* fragment in plasmid pGem-7Z, was used as a template to amplify the PCV1 ORF2.

In order to insert PCV2 ORF 2 into an appropriate ALVAC insertion vector : Primers JP787 (SEQ ID NO:12) and JP788 (SEQ ID NO:13) were used to amplify PCV1 ORF 2 from plasmid pPCV1 (see above) resulting in PCR J1315. Primer JP787 (SEQ ID NO:12) contains the 3' end of the H6 promoter from *EcoRV* and ORF 2 followed by a *SalI* site. The product of PCR J1315 was then digested with *EcoRV/SalI* and cloned as a ~750 bp fragment into a ~4.5 kb *EcoRV/SalI* fragment from pJP099 (see above in Example 1). The resulting plasmid was confirmed by sequence analysis and designated pJP113. The sequence of ORF 2 matches sequence available in GenBank, Accession Number U49186. The donor plasmid pJP113 (linearized with *NotI*) was used in an *in vitro* recombination (IVR) test to generate ALVAC recombinant vCP1621 (see Example 7).

Sequence of the primers:

JP787 (SEQ ID NO:12)

CAT-CAT-CAT-GAT-ATC-CGT-TAA-GTT-TGT-ATC-GTA-ATG-ACG-TGG-  
CCA-AGG-AGG-CG

JP788 (SEQ ID NO:13)

TAC-TAC-TAC-GTC-GAC-TTA-TTT-ATT-TAG-AGG-GTC-TTT-TAG-G

**Example 5 - CONSTRUCTION OF AN  
ALVAC DONOR PLASMID  
FOR PCV1 ORF2 AND ORF1**

Plasmid pPCV1 (see Example 4 above), containing the PCV1 genome as a *Pst*I fragment in plasmid pGem-7Z, was digested with *Pst*I, and a 1759 bp fragment was isolated and ligated.

Primers JP789 (SEQ ID NO:14) and JP790 (SEQ ID NO:15) were used to amplify PCV1 ORF1 from the 1759 bp ligated *Pst*I fragment (see above), resulting in PCR J1316. Primer JP789 (SEQ ID NO:14) contains the 3' end of the H6 promoter from *Nru*I and the 5' end of PCV1 ORF1. Primer JP790 (SEQ ID NO:15) contains the 3' end of PCV1 ORF1 followed by a *Sal*I site. The product of PCR J1316 (~1 Kb) was cloned into pCR2.1 (Invitrogen, Carlsbad, CA). The resulting plasmid was confirmed by sequence analysis and designated pJP114. The sequence of ORF1 matches sequence available in GenBank, Accession Number U49186. A ~970 bp *Nru*I/*Sal*I fragment was isolated from pJP114 and cloned into a ~4.5 kb *Nru*I/*Sal*I fragment from pJP099 (see Example 1), resulting in a plasmid which was confirmed by restriction analysis and designated pJP115. The donor plasmid pJP115 could be used in an *in vitro* recombination test (described in Example 7) to generate ALVAC recombinant expressing the PCV1 ORF1.

A ~838bp *Bam*HI/*Sal*I from pJP113 (see Example 4) was blunted using the Klenow fragment of DNA polymerase, and was cloned into the Klenow-blunted *Eco*RI site of pJP115. Clones were checked for orientation of insert by restriction analysis and a head-to-head orientation was chosen. This plasmid was confirmed by sequence analysis and designated pJP117. The donor plasmid pJP117 (linearized with *Not*I) was used in an *in vitro* recombination (IVR) test to generate the ALVAC recombinant vCP1622 (see Example 7).

Sequence of the primers:

JP789 (SEQ ID NO:14)

CAT-CAT-CAT-TCG-CGA-TAT-CCG-TTA-AGT-TTG-TAT-CGT-AAT-GCC-AAG-CAA-GAA-AAG-CGG

JP790 (SEQ ID NO:15)

TAC-TAC-TAC-GTC-GAC-TCA-GTA-ATT-TAT-TTT-ATA-TGG

**Example 6 - GENERATION OF ALVAC-PCV2 RECOMBINANTS**

Plasmids pJP102 (see Example 2 and Figure 2) and pJP107 (see Example 3 and Figure 5) were linearized with *NotI* and transfected into ALVAC infected primary CEF cells by using the calcium phosphate precipitation method previously described (Panicali and Paoletti, 1982 ; Piccini et al., 1987). Positive plaques were selected on the basis of hybridization to specific PCV2 radiolabeled probes and subjected to four sequential rounds of plaque purification until a pure population was achieved. One representative plaque from each IVR was then amplified and the resulting ALVAC recombinants were designated vCP1614 and vCP1615. The vCP1614 virus is the result of recombination events between ALVAC and the donor plasmid pJP102, and it contains the PCV2 ORF2 inserted into the ALVAC C6 locus. The vCP1615 virus is the result of recombination events between ALVAC and the donor plasmid pJP107, and it contains the PCV2 ORF2 and ORF1 inserted into the ALVAC C6 locus in a head-to-head orientation.

In a similar fashion, a recombinant ALVAC expressing only PCV2 ORF1 can be generated using the donor plasmid pJP105 described in Example 3.

Immunofluorescence. In order to determine if the PCV2 proteins were expressed in ALVAC recombinant infected Vero cells, immunofluorescence (IF) analysis was performed. Infected Vero cells were washed with PBS 24 hrs after infection (m.o.i. of approx. 10) and fixed with 95% cold acetone for 3 minutes at room temperature. Five monoclonal antibody (MAb) preparations (hybridoma supernatant) specific for PCV2 ORF1 (PCV2 199 1D3GA & PCV2 210 7G5GD) or ORF2 (PCV2 190 4C7CF, PCV2 190 2B1BC & PCV2 190 3A8BC) were used as the first antibody. These specific monoclonal antibodies were obtained from Merial-Lyon. Monoclonal antibodies can also be obtained following the teachings of documents cited herein, e.g. WO-A-99 18214, 1998, French applications Nos. 97/12382, 98/00873, 98/03707, filed October 3, 1997, January 22, 1998, and March 20, 1998, and WO99/29717, incorporated herein by reference. The IF reaction was performed as described by Taylor et al. (1990).

PCV2 specific immunofluorescence with the three ORF2-specific antibodies could be detected in cells infected with vCP1614 and cells infected with vCP1615. PCV2 specific immunofluorescence with the two ORF1-specific antibodies could be detected in cells infected with vCP1615 only. These results indicated that, as

expected, vCP1614 expresses only ORF2, whereas vCP1615 expresses both ORF1 and ORF2. No fluorescence was detected in parental ALVAC infected Vero cells, nor in uninfected Vero cells.

**Example 7 - GENERATION OF ALVAC-PCV1 RECOMBINANTS**

5 Plasmids pJP113 (see Example 4) and pJP117 (see Example 5) were linearized with *NotI* and transfected into ALVAC infected primary CEF cells by using the calcium phosphate precipitation method previously described (Panicali and Paoletti, 1982; Piccini et al., 1987). Positive plaques were selected on the basis of hybridization to specific PCV1 radiolabeled probes and subjected to four sequential  
10 rounds of plaque purification until a pure population was achieved. One representative plaque from each IVR was then amplified and the resulting ALVAC recombinants were designated vCP1621 and vCP1622. The vCP1621 virus is the result of recombination events between ALVAC and the donor plasmid pJP113, and it contains the PCV1 ORF2 inserted into the ALVAC C6 locus. The vCP1622 virus is the result  
15 of recombination events between ALVAC and the donor plasmid pJP117, and it contains the PCV1 ORF2 and ORF1 inserted into the ALVAC C6 locus in a head-to-head orientation.

In a similar fashion, a recombinant ALVAC expressing only PCV1 ORF1 can be generated using the donor plasmid pJP115 described in Example 5.

20 **Immunofluorescence.** In order to determine if the PCV1 proteins were expressed in ALVAC recombinant infected Vero cells, immunofluorescence (IF) analysis was performed. Infected Vero cells were washed with PBS 24 hrs after infection (m.o.i. of approx. 10) and fixed with 95% cold acetone for 3 minutes at room temperature. A specific anti-PCV1 pig polyclonal serum (Allan G. *et al.* Vet.  
25 Microbiol. 1999. 66: 115-123) was used as the first antibody. The IF reaction was performed as described by Taylor et al. (1990).

PCV1 specific immunofluorescence could be detected in cells infected with vCP1621 and cells infected with vCP1622. These results indicated that, as expected, vCP1621 and vCP1622 express PCV1-specific products. No fluorescence was  
30 detected with a PCV2-specific pig polyclonal serum in cells infected with vCP1621 and in cells infected with vCP1622. No fluorescence was detected in parental ALVAC infected Vero cells, nor in uninfected Vero cells.

**Example 8 - FORMULATION OF RECOMBINANT  
CANARYPOX VIRUSES WITH CARBOPOL™ 974P**

For the preparation of vaccines, recombinant canarypox viruses vCP1614 and vCP1615 (Example 6) can be mixed with solutions of carbomer. In the same fashion, recombinant canarypox viruses vCP1621 and vCP1622 (Example 7) can be mixed with solutions of carbomer. The carbomer component used for vaccination of pigs according to the present invention is the Carbopol™ 974P manufactured by the company BF Goodrich (molecular weight of # 3,000,000). A 1.5 % Carbopol™ 974P stock solution is first prepared in distilled water containing 1 g/l of sodium chloride.

This stock solution is then used for manufacturing a 4 mg/ml Carbopol™ 974P solution in physiological water. The stock solution is mixed with the required volume of physiological water, either in one step or in several successive steps, adjusting the pH value at each step with a 1N (or more concentrated) sodium hydroxide solution to get a final pH value of 7.3-7.4. This final Carbopol™ 974P solution is a ready-to-use solution for reconstituting a lyophilized recombinant virus or for diluting a concentrated recombinant virus stock. For example, to get a final viral suspension containing  $10^8$  pfu per dose of 2 ml, one can dilute 0,1 ml of a  $10^9$  pfu/ml stock solution into 1,9 ml of the above Carbopol™ 974P 4 mg/ml ready-to-use solution. In the same fashion, Carbopol™ 974P 2 mg/ml ready-to-use solutions can also be prepared.

**Example 9 - IMMUNIZATION OF PIGS  
AND SUBSEQUENT CHALLENGE**

**9.1. IMMUNIZATION OF 1 DAY-OLD PIGLETS**

Groups of piglets, caesarian-derived at Day 0, are placed into isolators. The piglets are vaccinated by intramuscular route at Day 2 with various vaccine solutions. Vaccine viral suspensions are prepared by dilution of recombinant viruses stocks in sterile physiological water (NaCl 0.9 %). Suitable ranges for viral suspensions can be determined empirically, but will generally range from  $10^6$  to  $10^{10}$ , and preferably about  $10^{10}$ , pfu/dose. Vaccine solutions can also be prepared by mixing the recombinant virus suspension with a solution of Carbopol™ 974P, as described in Example 8.

Piglets are vaccinated either with:

Recombinant virus vCP1614 (Example 2);

Recombinant virus vCP1615 (Example 3);



Recombinant virus vCP1614 mixed with Carbopol (4 mg/ml solution); or  
Recombinant virus vCP1615 mixed with Carbopol (4 mg/ml solution).

The viral suspensions contain  $10^8$  plaque forming units (pfu) per dose. Each viral suspension is injected by intramuscular route under a volume of 1 ml. The intramuscular injection is administered into the muscles of the neck.

Two injections of viral suspensions are administered at Day 2 and Day 14 of the experiment. A challenge is done on Day 21 by an oronasal administration of a viral suspension prepared from a culture of PCV-2 virulent strain. After challenge, piglets are monitored during 3 weeks for clinical signs specific of the post-weaning multisystemic syndrome. The following signs are scored :

Rectal temperature: daily monitoring for 2 weeks post-challenge, then 2 measures of rectal temperature during the third week.

Weight: piglets are weighed right before the challenge, and then weekly during the first 3 weeks post-challenge.

Blood samples are taken at Day 2, day 14, Day 21, Day 28, Day 35 and Day 42 of the experiment in order to monitor viremia levels and anti-PCV-2 specific antibody titers.

Necropsies: at Day 42, all surviving piglets are humanely euthanized and necropsied to look for specific PWMS macroscopic lesions. Tissue samples are prepared from liver, lymph nodes, spleen, kidneys and thymus in order to look for specific histological lesions.

## **9.2. IMMUNIZATION OF 5-7 WEEK-OLD PIGLETS**

5-7 week-old piglets, free of anti-PCV-2 specific maternal antibodies, are vaccinated by intramuscular route with various vaccine solutions. Vaccine viral suspensions are prepared by dilution of recombinant viruses stocks in sterile physiological water (NaCl 0.9 %). Vaccine solutions can also be prepared by mixing the recombinant virus suspension with a solution of Carbopol™ 974P, as described in Example 8.

Piglets are vaccinated either with:

Recombinant virus vCP1614 (Example 2);

Recombinant virus vCP1615 (Example 3);

Recombinant virus vCP1614 mixed with Carbopol (4 mg/ml solution); or

Recombinant virus vCP1615 mixed with Carbopol (4 mg/ml solution).

The viral suspensions contain  $10^8$  plaque forming units (pfu) per dose. Each viral suspension is injected by intramuscular route under a volume of 2 ml. The intramuscular injection is administered into the muscles of the neck.

Two injections of the viral suspensions are administered at Day 0 and Day 21 of the experiment. A challenge is done at Day 35 by an oronasal administration of a viral suspension prepared from a culture of PCV-2 virulent strain. After challenge, piglets are monitored during 8 weeks for clinical signs specific of the post-weaning multisystemic syndrome. The clinical monitoring is identical to the one described in Example 9.1. except that total duration of monitoring is 8 weeks instead of 3 weeks.

Necropsies are done throughout the experiment for piglets dying from the challenge and at the end of the experiment (Day 97) for all surviving piglets. Tissue samples are the same as described in Example 9.1.

### 9.3. IMMUNIZATION OF NEWBORN PIGLETS

Groups of 3 or 4 piglets, caesarian-delivered day 0 are placed into isolators.

Day 2 the piglets are vaccinated with  $10^8$  pfu of vCP1614, vCP1615 or parental ALVAC vector in 1 ml of PBS by intramuscular route on the side of the neck. A second injection of vaccine or placebo is administered at day 14. Vaccination with ALVAC recombinant is well tolerated by piglets and no evidence of adverse reaction to vaccination is noted. The piglets are challenged day 21 by oronasal administration of a PCV-2 viral suspension, 1 ml in each nostril. Day 45 necropsies are performed and samples of tissues are collected for virus isolation.

Necropsy results:

- PMWS is characterized generally by lymphadenopathy and more rarely by hepatitis or nephritis. So the gross findings in lymph nodes are scored for each piglet in the following manner : 0 = no visible enlargement of lymph nodes ; 1 = mild lymph nodes enlargement, restricted to bronchial lymph nodes ; 2 = moderate lymph nodes enlargement, restricted to bronchial lymph nodes ; 3 = severe lymph nodes enlargement, extended to bronchial, submandibular prescapular and inguinal lymph nodes.

26

<u>Groups</u>	<u>Scores</u>
<b>vCP 1614</b>	0.5
	0.0
	0.0
	1.0
mean	0.38
standard deviation	0.48
<b>vCP 1615</b>	0.0
	0.5
	0.5
	1.0
mean	0.5
standard deviation	0.41
<b>Controls</b>	2.0
	2.5
	2.5
	2.5
mean	2.38
standard deviation	0.25

Bronchial lymphadenopathy for PCV-2 is a prominent gross finding. A significant reduction of the lymph nodes lesion in relation to control group is observed after immunization with vCP 1614 and vCP 1615 ( $p \leq 0.05$ ).

5

\* \* \*

Having thus described in detail preferred embodiments of the present invention, it is to be understood that the invention defined by the appended claims is not to be limited to particular details set forth in the above description as many apparent variations thereof are possible without departing from the spirit or scope of the present invention.

10

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**WHAT IS CLAIMED IS:**

1. A recombinant virus comprising DNA from porcine circovirus 2.
2. The recombinant virus of claim 1 which is a poxvirus.
3. The recombinant poxvirus of claim 2 which is an avipox virus.
- 5 4. The recombinant avipox virus of claim 3 which is ALVAC.
5. The recombinant ALVAC virus of claim 4, wherein the DNA from porcine circovirus 2 codes for and is expressed as the porcine circovirus major capsid protein or an epitope of interest.
6. The recombinant ALVAC virus of claim 4, wherein the DNA from porcine  
10 circovirus 2 comprises of the open reading frame 2 (ORF2) of porcine circovirus 2.
7. The recombinant ALVAC virus of claim 4, wherein the DNA from porcine circovirus 2 comprises of the open reading frame 1 (ORF1) of porcine circovirus 2.
- 15 8. The recombinant ALVAC virus of claim 4, wherein the DNA from porcine circovirus 2 comprises of the open reading frames 1 and 2 (ORF1 and 2) of porcine circovirus.
9. The recombinant ALVAC virus of claim 4 which is vCP1614 or vCP1615.
10. An immunological composition for inducing an immunological response in a host  
20 inoculated with the immunological composition, the immunological composition comprising a carrier and the recombinant virus of claim 1.
11. An immunological composition for inducing an immunological response in a host inoculated with the immunological composition, the immunological composition comprising a carrier and the recombinant virus of claim 5.
- 25 12. An immunological composition for inducing an immunological response in a host inoculated with the immunological composition, the immunological composition comprising a carrier and the recombinant virus of claim 6.
13. An immunological composition for inducing an immunological response in a host inoculated with the immunological composition, the immunological composition  
30 comprising a carrier and the recombinant virus of claim 7.
14. An immunological composition for inducing an immunological response in a host inoculated with the immunological composition, the immunological composition comprising a carrier and the recombinant virus of claim 8.

15. An immunological composition for inducing an immunological response in a host inoculated with the immunological composition, the immunological composition comprising a carrier and the recombinant virus of claim 9.
16. A method for inducing an immunological response in a host comprising  
5 administering to the host the immunological composition of claim 11.
17. A method for inducing an immunological response in a host comprising administering to the host the immunological composition of claim 12.
18. A method for inducing an immunological response in a host comprising administering to the host the immunological composition of claim 13.
- 10 19. A method for inducing an immunological response in a host comprising administering to the host the immunological composition of claim 14.
20. A method for inducing an immunological response in a host comprising administering to the host the immunological composition of claim 15.

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HindIII (1)

1 AAGCTTCTATCAAAAGTCTTAATGAGTTAGGTGTAGATAGTATAGATATTACTACAAAGGTATTCATATT  
71 TCCTATCAATTCTAAAGTAGATGATATTAATAACTCAAAGATGATGATAGTAGATAATAGATACGCTCAT  
143 ATAATGACTGCAAAATTTGGACGGTTCACATTTTAATCATCACGCGTTCATAAGTTTCAACTGCATAGATC  
211 AAAATCTCACTAAAAAGATAGCCGATGTATTTGAGAGAGATTGGACATCTAACTACGCTAAAGAAATTAC  
281 AGTTATAAATAATACATAATGGATTTTGTATCATCAGTTATATTTAACATAAGTACAATAAAAAGTATT  
351 AAATAAAAATACTTACTTACGAAAAAATGTCATTATTACAAAACTATATTTTACAGAACAATCTATAGT  
138 Met Ser LeuLeuGlnLysLeuTyrPheThr GluGlnSer IleVal  
421 AGAGTCCCTTTAAGAGTTATAATTTAAAAGATAACCATAATGTAATATTTACCACATCAGATGATGATACT  
158 GluSer PheLysSer TyrAsnLeuLysAspAsnHisAsnVal IlePheThr Thr Ser AspAspAspThr  
491 GTTGTAGTAATAAATGAAGATAATGTACTGTATCTACAAGATTATATCATTTTGATAAAATTCTGTTTT  
398 ValValVal IleAsnGluAspAsnValLeuLeuSer ThrArgLeuLeuSer PheAspLys IleLeuPheP  
561 TTAATCCTTTAATAACGGTTTATCAAAATACGAACTATTAGTGATACAATATTAGATATAGATACTCA  
628 haAsnSer PheAsnAsnGlyLeuSerLysTyrGluThr IleSerAspThr IleLeuAsp IleAspThr Hi  
631 TAATTATTATATACCTAGTCTCTCTCTTTGTAGATATTCTAAAAAAGAGCGGTGATTTAGAAITA  
858 sAsnTyrTyr IleProSer Ser Ser LeuLeuAsp IleLeuLysLysArgAlaCysAspLeuGluLeu  
701 GAAGATCTAAATTATGCGTTAATAGGAGACAATAGTAACCTTATATTATAAAGATATGACTTACATGAATA  
1098 GluAspLeuAsnTyrAlaLeu IleGluAspAsnSerAsnLeuTyrTyrLysAspMetThr TyrMetAsnA  
771 ATTGGTTATTTACTAAAGGATTATTAGATTACAAGTTTGTATTATTGCGCGATGTAGATAAATGTTACAA  
1328 snTrpLeuPheThr LysGluLeuLeuAspTyrLysPheValLeuLeuArgAspValAspLysCysTyrLy  
NruI (880) NdeI (901)  
841 ACAGTATATAAAAAAGAACTACTATAATAGATATAATACATCGCGATAACAGACAGTATAACATATGGGTT  
1558 sGlnTyrAsnLysLysAsnThr IleIleAspIleIleHisArgAspAsnArgGlnTyrAsn IleTrpVal  
911 AAAAATGTTATAGAATACTGTCTCTCTGGCTATATATTATGGTTACATGATCTAAAAGCCGCTGCTGAAG  
1798 LysAsnVal IleGluTyrCysSer ProGlyTyr IleLeuTrpLeuHisAspLeuLysAlaAlaAlaGluA  
981 ATGATTGGTTAAGATACGATAACCGTATAAACGAATTATCTGCGGATAAATTATACACTTTTCGAGTTTCAT  
2028 spAspTrpLeuArgTyrAspAsnArg IleAsnGluLeuSer AlaAspLysLeuTyrThr PheGluPheIle  
1051 AGTTATATTAGAAAATAATATAAACATTTACGAGTAGGTACATAATTGTACATCCAAACAAGATAATA  
2258 eVal IleLeuGluAsnAsn IleLysHisLeuArgValGlyThr IleIleValHisProAsnLys IleIle  
1121 GCTAATGGTACATCTAATAATATACTTACTGATTTTCTATCTTACGTAGAAGAACTAATATATCATCATA  
2498 AlaAsnGlyThr SerAsnAsn IleLeuThrAspPheLeuSer TyrValGluGluLeuIleTyrHisHisA  
EcoRI (1223)  
1191 ATTCACTCTATAATATTTGGCCGGATATTTTTTAGAATCTTTGAGACCACTATTTTATCAGAAATTTATTTT  
2728 snSer Ser-IleIleLeuAlaGlyTyrPheLeuGluPhePheGluThr Thr IleLeuSer GluPheIleSe  
1261 TTCATCTTCTGAATGGGTAATGAATAGTAACCTGTTTAGTACACCTGAAAACAGGGTATGAAGCTATACTC  
2958 rSerSerSer GluTrpValMetAsnSerAsnCysLeuValHisLeuLysThr GlyTyrGluAlaIleLeu  
1331 TTTGATGCTAGTTTATTTTCCAACTCTCTACTAAAAGCAATTATGTAAAATATTGGACAAAGAAAACCT  
3198 PheAspAlaSer LeuPhePheGlnLeuSer Thr LysSerAsnTyrValLysTyrTrpThr LysLysThrL  
1401 TGCAGTATAAGAACCTTTTTTAAAGACGGTAAACAGTTAGCAAAATATATAATTAAAGAAAGATAGTCAGGT  
3428 euGlnTyrLysAsnPhePheLysAspGlyLysGlnLeuAlaLysTyr IleIleLysLysAspSer GlnVa



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1471 GATAGATAGAGTATGTTATTTACACGCAGCTGTATATAATCACGTAACCTTACTTAATGGATACGTTTAAA  
365P I I I eAspArgVal CysTyrLeuHl sAl sAl sVal TyrAsnHl sVal Thr TyrLeuMetAspThr PheLys

1541 ATTCCCTGGTTTTGATTTTAAATTTCTCCGGAATGATAGATATACTACTGTTTGGGAATATTGCATAAGGATA  
389P I I eProGlyPheAspPheLysPheSer GlyMet I I eAsp I I eLeuLeuPheGly I I eLeuHl sLysAspA

1611 ATGAGAATATATTTTATCCGAAACGTGTTTCTGTAACATAATATAATATCAGAATCTATCTATGCAGATTT  
412P snGluAsn I I ePheTyrProLysArgValSerValThrAsn I I e I I eSer GluSer I I eTyrAl sAspPh

1661 TTACTTTTATATCAGATGTTAATAAATTCAGTAAAAAGATAGAAATATAAACTATGTTTCTTACTACTCGCA  
435P eTyrPhe I I eSerAspValAsnLysPheSerLysLys I I eGluTyrLysThrMetPhePro I I eLeuAl s

1751 GAAACTACTATCCAAAAGGAAGGCCCTATTTTACACATACATCTAACGAAGATCTTCTGTCTATCTGTT  
459P GluAsnTyrTyrProLysGlyArgProTyrPheThrHl sThrSerAsnGluAspLeuLeuSer I I eCysL

1821 TATGCGAAGTAACAGTTTGTAAAGATATAAAAAATCCATTATTATATTCTAAAAAGGATATATCAGCAA  
482P euCysGluValThrValCysLysAsp I I eLysAsnProLeuLeuTyrSerLysLysAsp I I eSerAl sLys

1891 ACGATTCATAGGTTTATTTACATCTGTGATATAAAATACGGCTGTTGAGTTAAGAGGATATAAAATAAGA  
505P sArgPhe I I eGlyLeuPheThrSerValAsp I I eAsnThrAl sValGluLeuArgGlyTyrLys I I eArg

1961 GTAATAGGATGTTTAGAATGGCCCTGAAAAGATAAAAAATTTTAATTTCTAATCTACATACATTAGATTAT  
529P Val I I eGlyCysLeuGluTyrProGluLys I I eLys I I ePheAsnSerAsnProThrTyr I I eArgLeuL

2031 TACTAACAGAAAGACGTTTATAGATATTTCTACATTCCTATCTGCTTAAATTTAATATAACAGAGGATATAGC  
552P euLeuThrGluArgArgLeuAsp I I eLeuHl sSerTyrLeuLeuLysPheAsn I I eThrGluAsp I I eAl

2101 TACCAGAGATGGAGTCAGAAATAATTTACCTATAATTTCTTTTATCGTCAGTTATTTGTAGATCGTATACT  
575P sThrArgAspGlyValArgAsnAsnLeuPro I I e I I eSerPhe I I eValSerTyrCysArgSerTyrThr

NdeI (2188)

2171 TATAAATTACTAAATGGCCATATGTACAAATTCGTGTAAGATAACAAAGTGTAATATAATCAGGTAATAT  
599P TyrLysLeuLeuAsnCysHl sMetTyrAsnSerCysLys I I eThrLysCysLysTyrAsnGlnVal I I eT

2241 ATAATCCTATATAGGAGTATATATAATTGAAAAAGTAAAATATAAATCATATAAATGAACGAAATAT  
622P yrAsnPro I I e . . .

2311 CAGTAATAGACAGGAACCTGGCAGATTCTTCTTCTAATGAAGTAAGTACTGCTAAATCTCCAAAATTAGAT

2381 AAAAATGATACAGCAATAACAGCTTCATTCAACGAATTACCTTTTAAATTTTTCAGACACACCTTATTAC

2451 AAACCTAATAAGTCAGATGATGAGAAAGTAAATATAAATTTAAGTTATGGGTATAATATAATAAGATTC

2521 ATGATATTAAATAATTTACTTAAACGATGTTAATAGACTTATTCATCAACCCCTTCAAACCTTTCTGGATA

2591 TTATAAAATACCAGTTAATGATATTAAAATAGATTGTTTAAAGAGATGTAAATAATTATTTGGAGGTAAAG

2661 GATATAAAATTAGTCTATCTTTTACATGGAAATGAATTACCTAATATTAAATAATTATGATAGGAATTTT

2731 TAGGATTTACAGCTGTTATATGTATCAACAATACAGGCAGATCTATGGTTATGGTAAAACACTGTAACGG

2801 GAAGCAGCATTCTATGGTAACTGGCCTATGTTTAAATAGCCAGATCATTTTACTCTATAAACATTTTACCA

BamHI (2880)

2871 CAAATAATAGGATCCTCTAGATATTTAATATTATATCTAACAACAACAAAAAATTTAACGATGTATGGC

2941 CAGAAGTATTTTCTACTAATAAAGATAAAGATAGTCTATCTTATCTACAAGATATGAAAGAAGATAATCA

HindIII (3058)

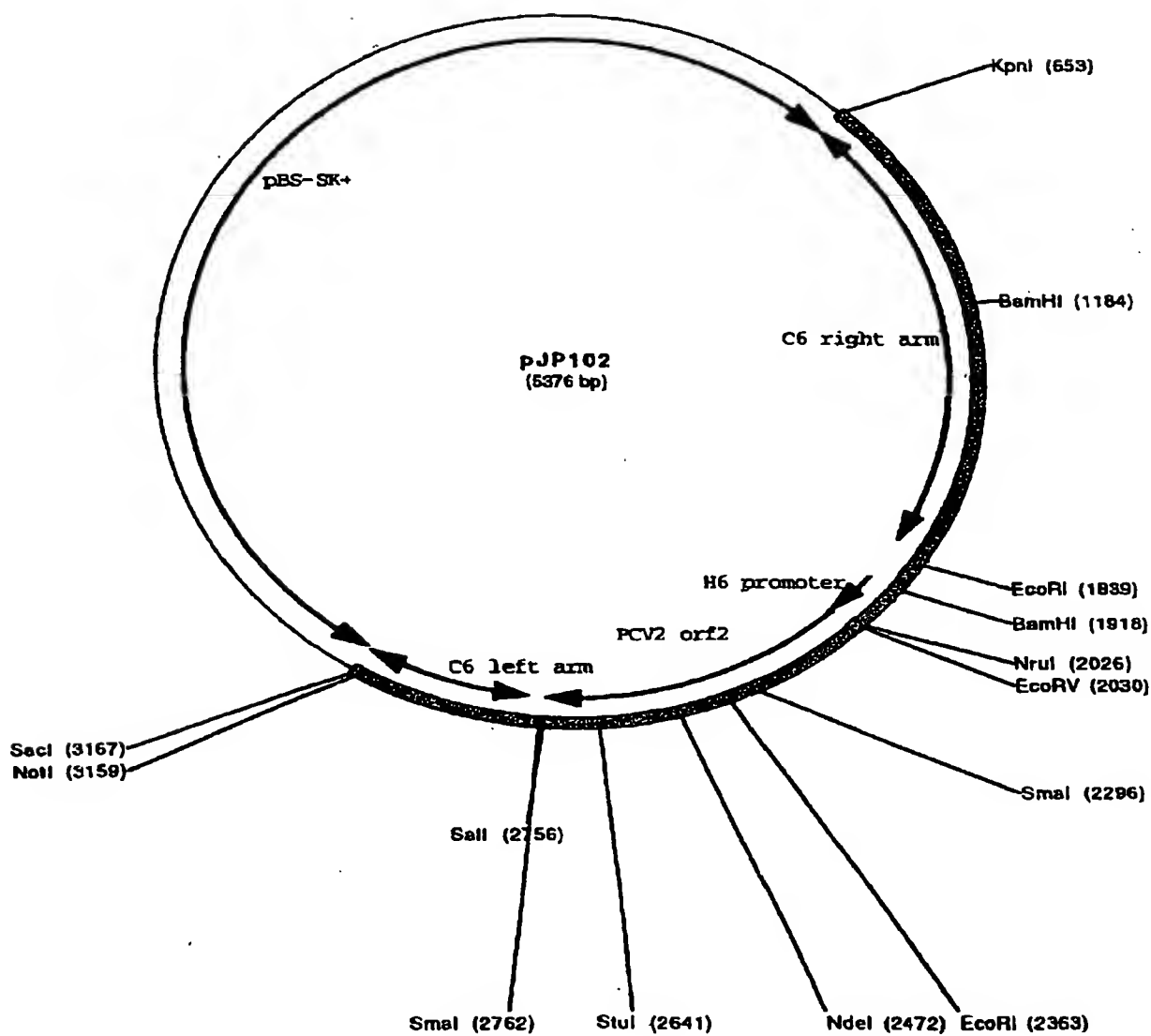
3011 TTTAGTAGTAGCTACTAATATGGAAAGAAATGTATACAAAAACGTGGAAGCTTTTATATTAAATAGCATA

3081 TTAGTAGAAGATTTAAAATCTAGACTTAGTATAACAAAACAGTTAAATGCCAATATCGATTCTATATTTT

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3151 ATCATAACAGTAGTACATTAATCAGTGATATACTGAAACGATCTACAGACTCAACTATGCAAGGAATAAG  
3221 CAATATGCCAATTATGTCCTAATATTTTAACTTTAGAACTAAAACGTTCTACCAATACTAAAAATAGGATA  
3291 CGTGATAGGCTGTTAAAAGCTGCAATAAATAGTAAGGATGTAGAAGAAATACTTTGTTCTATACCTTCGG  
3361 AGGAAAGAACTTTAGAACAACTTAAGTTTAATCAAACCTTGATTTATGAACACTATAAAAAAATTATGGA  
3431 AGATACAAGTAAAAGAATGGATGTTGAATGTCGTAGTTTAGAACATAACTATACGGCTAACTTATATAAA  
3501 GTGTACGGACAAAACGAATATATGATTACTTATATACTAGCTCTCATAAGTAGGATTAAATAATATTATAG  
3571 AAACTTTAAATATAAATCTGGTGGGCTAGACGAATCTACAATACGTAATATAAATTATATAATTTCACA  
3641 AAGAACAAAAAAAATCAAGTTTCTAATACCTTATAGATAAACTATATTTTTTACCCTGA

Fig 2



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KpnI (1)

1 GGTACCTTCATAAATACAAGTTTGATTAACTTAAGTTGTTCTAAAGTTCTTTCTCCGAAGGTATAGAA  
71 CAAAGTATTTCTTCTACATCCTTACTATTATTGCAGCTTTTAACAGCCTATCACGTATCCTATTTTCTAG  
141 TATTGGTAGAACGTTTTAGTTCTAAAGTTAAAATATTAGACATAATTGGCATATTGCTTATTCTCTGCAT  
211 AGTTGAGTCTGTAGATCGTTTTAGTATATCACTGATTAAATGTACTACTGTTATGATGAAATATAGAATCG  
281 ATATTGGCATTTAACTGTTTTGTTATACTAAGTCTAGATTTTAAATCTTCAGTAATATGCTATTTAATA  
351 TAAAAGCTTCCACGTTTTTGTATACATTTCTTTCCATATTAGTAGCTACTACTAAATGATTATCTTCTTT  
421 CATATCTTGTAGATAAGATAGACTATCTTTATCTTTATTAGTAGAAAATACTTCTGGCCATACATCGTTA

BamHI (532)

491 AATTTTTTTGTTGTTGTTAGATATAATATTAAATATCTAGAGGATCCTATTATTTGTGGTAAAATGTTTA  
561 TAGAGTAAAATGATCTGGCTATTAAACATAGGCCAGTTACCATAGAATGCTGCTTCCCGTTACAGTGT  
631 TACCATAACCATAGATCTGCTGTATTGTTGATACATATAACAGCTGTAAATCCTAAAAAATTCCTATCA  
701 TAATTATTAAATATTAGGTAATTCATTTCCATGTGAAAGATAGACTAATTTTATATCTTTACCTCCAAAT  
771 AATTATTTACATCTCTTAAACAATCTATTTTAAATATCATTAAGTGGTATTTTATAATATCCAGAAAGGTT  
841 TGAAGGGGTGTAGTGAATAAGTCTATTAACATCGTTAAGTAAATTTAATATCATGAATCTTTATTATA  
911 TTATACCCATAAGTTAAATTTATATTTACTTTCTCATCATCTGACTTAGTTAGTTTGTATAAAGGTGTGT  
981 CTGAAAAAATTTAAAGGTAATTCGTTGAATGAAGCTGTATTTGCTGTATCATTTTTATCTAATTTTGGAG  
1051 ATTTAGCAGTACTTACTTCATTAGAAGAAGATCTGCCAGTTCTGTCTATTACTGATATTTTCGTTTCAT

EcoRI (1)

1121 TATTATATGATTTATATTTACTTTTTCAATTATATATACTCATTGACTAGTTAATCAATAAAAAGAAT  
1191 TCCTGCAGCCCTGCAGCTAATTAATTAAGCTACAAATAGTTTCGTTTTACCTTGTTCTAATAACTAATTA

BamHI (1266)

1261 ATTAAGGATCCCCAGCTTCITTAATCTATACITAAAAAGTGAAAATAAATACAAAGGTTCTTGAGGGTT

EcoRV (1378)

NruI (1374)

1331 GTGTTAAATTGAAAGCGAGAAATAATCATAAATTATTTTCTATATCGCGATATCGTTAAGTTGTATCGT  
1401 AATGACGTATCCAAGGAGGCGTTACCGCAGAAGAAGACACCGCCCCCGCAGCCATCTTGGCCAGATCCTC  
1471 CGCCGCGCGCCCTGGCTCGTCCACCCCGCCACCGCTACCGTTGGAGAAGGAAAAATGGCATCTTCAACA  
241 ArgArgArgProTrpLeuValHisProArgHisArgTyrArgTrpArgArgLysAsnGlyIlePheAsnT

1541 CCCGCTCTCCCGACCTTCGGATATACTGTCAAGCGTACCACAGTCACAACGCCCTCCTGGGCGGTGGA  
471 hrArgLeuSerArgThrPheGlyTyrThrValLysArgThrThrValThrThrProSerTrpAlaValAs

SmaI (1644)

1611 CATGATGAGATTTAAAATTGACGACTTTGTTCCCGCGGAGGGGGACCAACAAAATCTCTATACCCCTT  
701 pMetMetArgPheLysIleAspAspPheValProProGlyGlyGlyThrAsnLysIleSerIleProPhe

## EcoRI (1711)

1681 GAATACTACAGAATAAGAAAGGTTAAGGTTGAATTCGGCCCTGCTCCCCATCACCAGGGTGATAGGG  
94▶ GluTyrTyrArgIleArgLysValLysValGluPheTrpProCysSerProIleThrGlnGlyAspArgG

## NdeI

1751 GAGTGGGCTCCACTGCTGTTATTCTAGATGATAACTTTGTAACAAAGGCCACAGCCCTAACCTATGACCC  
117▶ IyValGlySerThrAlaValIleLeuAspAspAsnPheValThrLysAlaThrAlaLeuThrTyrAspPr

1821 ATATGTAACTACTCCTCCCGCCATACAATCCCCAACCCCTTCTCTACCACTCCCGTTACTTCACACCC  
140▶ oTyrValAsnTyrSerSerArgHisThrIleProGlnProPheSerTyrHisSerArgTyrPheThrPro

1891 AAACCTGTCTTGAAGTCCACTATTGATTACTTCCAACCAAATAACAAAAGGAATCAGCTTTGGCTGAGAC  
164▶ LysProValLeuAspSerThrIleAspTyrPheGlnProAsnAsnLysArgAsnGlnLeuTrpLeuArgL

## StuI (1889)

1961 TACAAACCTCTGGAAATGTGGACCACGTAGGCCTCGGCGCTGCGTTGAAAACAGTAAATACGACCAGGA  
187▶ euGlnThrSerGlyAsnValAspHisValGlyLeuGlyAlaAlaPheGluAsnSerLysTyrAspGlnAs

2031 CTACAATATCCGTGTAACCATGTATGTACAATTCAGAGAATTTAATCTTAAAGACCCCCCACTTAAACCC  
210▶ pTyrAsnIleArgValThrMetTyrValGlnPheArgGluPheAsnLeuLysAspProProLeuLysPro

## SmaI (2110)

## SalI (2104)

2101 TAAGTCGACCCCGGGTTTTTATAGCTAATTAGTCATTTTTTCGTAAGTAAGTATTTTTATTTAATACTTT  
2171 TTATTGTAAGTATGTTAAATATAACTGATGATAACAAATCCATTATGTATTATTTATAACTGTAATTTT  
2241 TTTAGCGTAGTTAGATGTCCAATCTCTCTCAAATACATCGGCTATCTTTTTAGTGAGATTTTGATCTATG  
2311 CAGTTGAAACTTAATGAACGCGTGATGATTAATATGTGAACCGTCCAAATTTGCAGTCATTATATGAGCGT  
2381 ATCTATTATCTACTATCATCATCTTTGAGTTATTAATATCATCTACTTTAGAATTGATAGGAAATATGAA

## SacI (2515)

## NotI (2507)

2451 TACCTTTGTAGTAATATCTATACTATCTACACCTAACTCATTAAAGACTTTTGATAGGCGGCCGAGCTC

Fig. 3B

Fig. 4

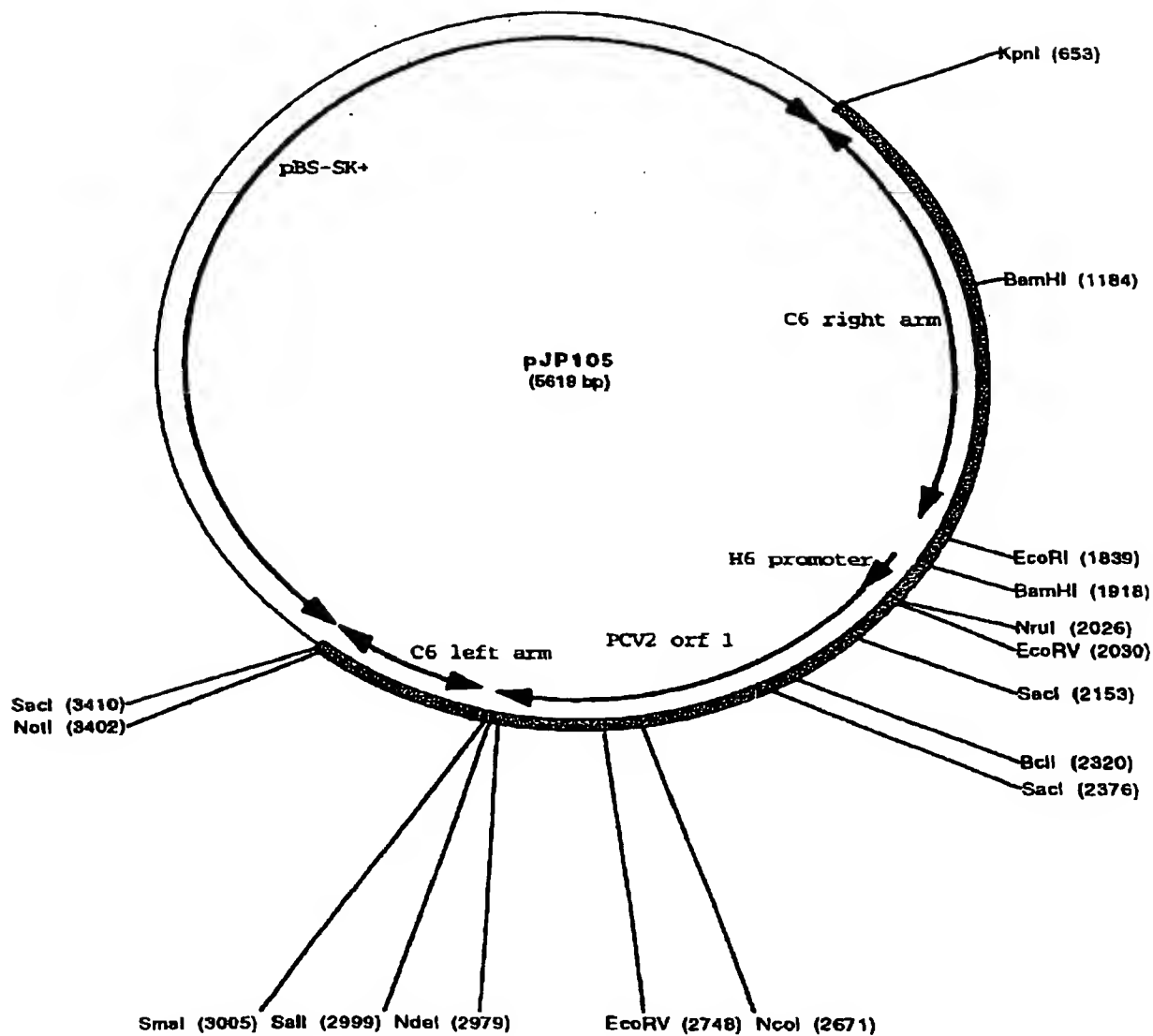


Fig 6A

KpnI (1)

1 GGTACCTTCATAAATACAAGTTTGATTAACTTAAGTTGTTCTAAAGTTCITTCCTCCGAAGGTATAGAA  
71 CAAAGTATTTCTTCTACATCCTTACTATTTATTCAGCTTTTAACAGCCTATCACGTATCCTATTTTATG  
141 TATTGGTAGAACGTTTTAGTTCTAAAGTTAAATATTAGACATAATTGGCATATTGCTTATTCCTTGCAT  
211 AGTTGAGTCTGTAGATCGTTTCAGTATATCACTGATTAATGTACTACTGTTATGATGAAATATAGAATCG  
281 ATATTGGCATTTAAGTGTMTTGTATATAAGTCTAGATTTTAAATCTTCTAGTAATATGCTATTTAATA  
351 TAAAAGCTTCCACGTTTTTGTATACATTTCTTTCCATATTAGTAGCTACTACTAAATGATTATCTTCTTT  
421 CATATCTTGTAGATAAGATAGACTATCTTTATCTTTATTAGTAGAAAATACTTCTGGCCATACATCGTTA

BamHI (532)

491 AATTTTTTTGTTGTTGTTAGATATAATATTAATATCTAGAGGATCCTATTATTGTTGGTAAAATGTTTA  
561 TAGAGTAAAATGATCTGGCTATTAAACATAGGCCAGTTACCATAGAAATGCTGCTTCCCGTTACAGTGTTT  
632 TACCATAACCATAGATCTGCCCTGTATTGTTGATACATATAACAGCTGTAAATCCTAAAAAATTCCTATCA  
701 TAATTATTAATATTAGGTAATTCATTTCCATGTGAAGATAGACTAATTTATATCCTTTACCTCCAAAT  
771 AATTATTACATCTCTTAACAATCTATTTAATATCATTAAGTGGTATTTTATAATATCCAGAAAGGTT  
841 TGAAGGGGTTGATGGAATAAGTCTATTAACATCGTTAAGTAAATTTAATATCATGAATCTTTATTATA  
911 TTATACCCATAAGTTAAATTTATATTTACTTTCTCATCATCTGACTTAGTTAGTTTGTAAATAAGGTGTGT  
981 CTGAAAAAATTAAAAGGTAATTCGTTGAATGAAGCTGATTGCTGTATCATTTTTATCTAATTTTGGAG  
1051 ATTTAGCAGTACTTACTTCATTAGAGAAGAATCTGCCAGTTCCTGTCTATTACTGATATTTCTGTTTCAT  
1121 TATTATATGATTTATATTTTACTTTTTCAATTATATATACTCAATTGACTAGTTAATCAATAAAAAGAAT  
1191 TTCGACTTAGGGTTTAAAGTGGGGGCTCTTTAAGATTAAATTTCTCTGAATTGTACATACATGGTTACACGG  
233 4 ProLysLeuProProAspLysLeuAsnPheGluArgPheGlnValTyrMetThrValArgI

SstI (1306)

1261 ATATTGTAGTCTCGGTCTATTTACTGTTTTGGAACGCAGCGCCGAGGCTACGTGGTCCACATTTCCAG  
212 4 LeAsnTyrAspGlnAspTyrLysSerAsnGluPheAlaAlaGlyLeuGlyValHisAspValAsnGlySe  
1331 AGGTTGTAGTCTCAGCCAAAGCTGATTCCTTTTGTATTGTTGGTGGAAAGTAATCAATAGTGGAGTCAAG  
189 4 r ThrGlnLeuArgLeuTrpLeuGlnAsnArgLysAsnAsnProGlnPheTyrAspIleThrSerAspLeu  
1401 AACAGGTTTGGGTGTGAAGTAACGGGAGTGGTAGGAGAAGGTTGGGGGATTGTATGCGGGAGGAGTAG  
166 4 ValProLysProThrPheTyrArgSerHisTyrSerPheProGlnProIleThrHisArgSerSerTyrA

NdeI (1475)

1471 TTTACATATGGGTCATAGGTTAGGGCTGTGGCCTTTGTTACAAAGTTATCATCTAGAATAACAGCAGTGG  
142 4 enValTyrProAspTyrThrLeuAlaThrAlaLysThrValPheAsnAspAspLeuIleValAlaThrSe

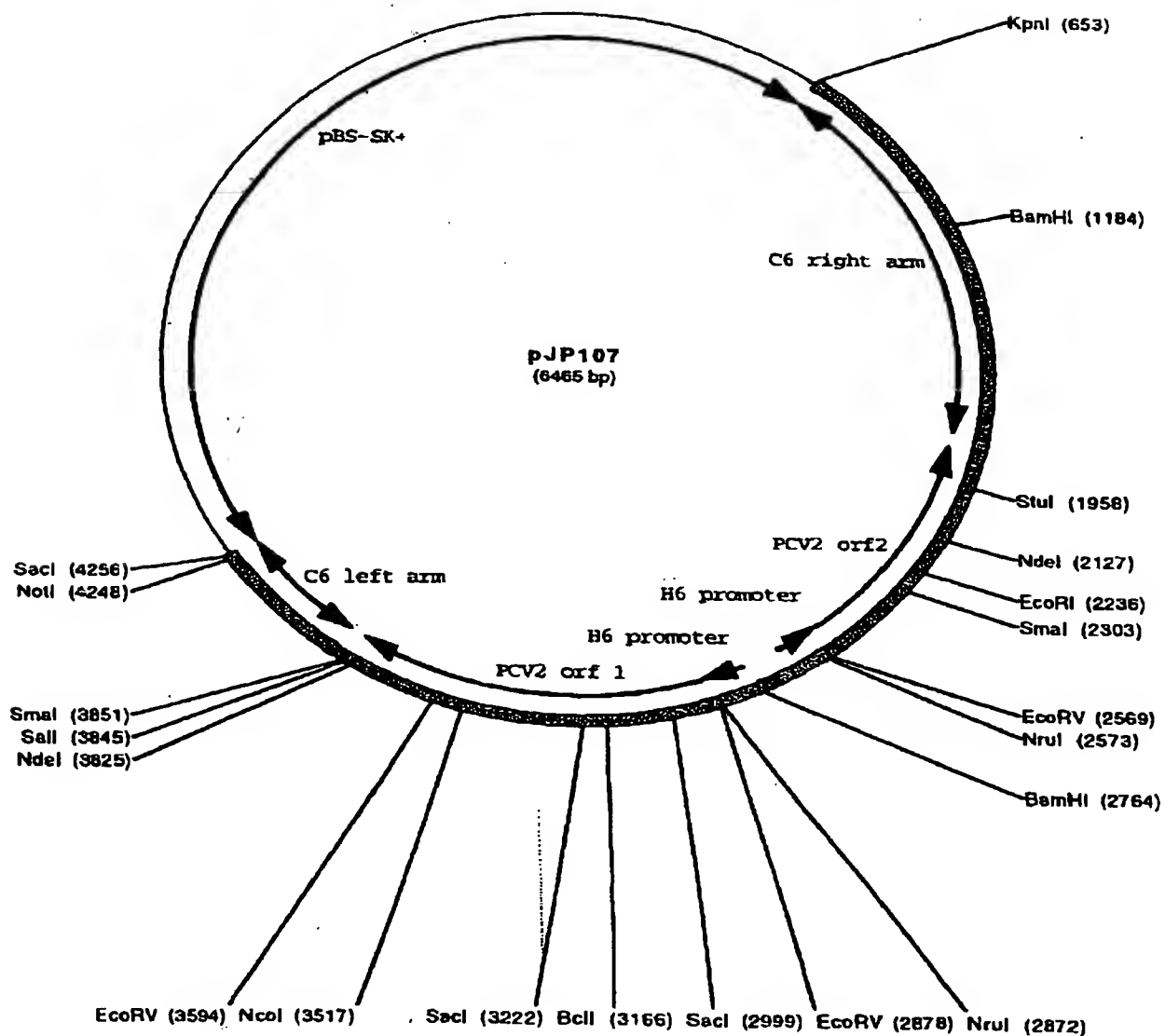
EcoRI (1584)

1541 AGCCCACTCCCTATCACCTGGGTGATGGGGAGCAGGGCCAGAAATTCACCTTAACCTTTCTTATTCT  
119 4 r GlyValGlyArgAspGlyGlnThrIleProSerCysProTrpPheGluValLysValLysArgIleArg

SmaI (1651)

1611 GTAGTATTCAAAGGGTATAGAGATTTTGTGGTCCCCCTCCCGGGGGAACAAAGTCGTCAATTTTAAAT  
96 4 TyrTyrGluPheProIleSerIleLysAsnThrGlyGlyGlyProProValPheAspAspIleLysPheA

9/11  
Fig 5





168- CTCATCATGTCCACCGCCAGGAGGGCGTGTGTGACTGTGGTACGCTTGACAGTATATCCGAAGGTGCGGG  
724 r gMetMetAspValAlaIaIrpSerProThrThrValThrThrArgLysValThrTyrGlyPheThrArgSe

1751 AGAGCGGGGTGTGAAGATGCCATTITTCCTTCTCCAACGGTAGCGGTGGCGGGGTGGACGAGCCAGGG  
494 r LeuArgThrAsnPheIleGlyAsnLysArgArgTrpArgTyrArgHisArgProHisValLeuTrpPro

1821 GCGGCGGCGGAGGATCTGGCCAAGATGGCTGCGGGGGCGGTGTCTTCTCTGCGGTAACGCGCTCCTTGGGA  
254 ArgArgArgLeuIleGlnGlyLeuHisSerArgProArgHisArgArgArgArgTyrArgArgArgProT

2941 CGATATCCATTGACTGTAGAGACTAAAGGTGGAACTGTACCTTTTTTGGCCCGCAGTATTCTGATTACCA  
232P A r g T y r P r o L e u T h r V a l | G l u T h r L y s G l y G l y T h r V a l | P r o P h e L e u A l a A r g S e r | l e L e u l e T h r S

11/11

3011 GCAATCAGACCCCGTTGGAATGGTACTCCTCAACTGCTGTCCAGCTGTAGAAGCTCTCTATCGGAGGAT  
255▶ erAsnGlnThrProLeuGluTrpTyrSerSerThrAlaValProAlaValGluAlaLeuTyrArgArgI

3081 TACTTCCTTGGTATTTTGAAGAATGCTACAGAACAATCCACGGAGGAAGGGGGCCAGTTCGTCACCCCTT  
278▶ eThrSerLeuValPheTrpLysAsnAlaThrGluGlnSerThrGluGluGlyGlyGlnPheValThrLeu

NdeI (3179)

SmaI (3199)

Sall (3193)

3151 TCCCCCCCATGCCCTGAATTTCCATATGAAATAAATTACTGAGTCGACCCCGGGTTTTTATAGCTAATTA  
302▶ SerProProCysProGluPheProTyrGluIleAsnTyr

3221 GTCATTTTTTCGTAAGTAAGTATTTTTATTTAATACTTTTTATTGTACTTATGTTAAATATAACTGATGA

3291 TAACAAAATCCATTATGTATTATTTATAACTGTAATTTCTTTAGCGTAGTTAGATGTCCAATCTCTCTCA

3361 AATACATCGGCTATCTTTTTAGTGAGATTTTGATCTATGCAGTTGAAACTTATGAACGCGTGATGATTAA

3431 AATGTGAACCGTCCAAATTTGCAGTCATTATATGAGCGTATCTATTATCTACTATCATCATCTTTGAGTT

3501 ATTAATATCATCTACTTTAGAATTGATAGGAAATATGAATACCTTTGTAGTAATATCTATACTATCTACA

SacI (3804)

NotI (3596)

3571 CCTAACTCATTAAGACTTTTGATAGGCGGCCGCGAGCTC

## SEQUENCE LISTING

## (1) GENERAL INFORMATION:

(i) APPLICANT: Bublot, Michel  
Perez, Jennifer M.  
Charreyre, Catherine E.

(ii) TITLE OF INVENTION: Porcine Circovirus 2 Recombinant  
Poxvirus

(iii) NUMBER OF SEQUENCES: 13

## (iv) CORRESPONDENCE ADDRESS:

(A) ADDRESSEE: ~~Virogenetics Inc.~~ *Frommer Lawrence & Haug LLP*  
(B) STREET: ~~465 Jordan Road~~ *745 Fifth Avenue*  
(C) CITY: ~~Fremont~~ *NY*  
(D) STATE: ~~NY~~ *NY*  
(E) COUNTRY: USA  
(F) ZIP: ~~12180~~ *10151*

## (v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk  
(B) COMPUTER: IBM PC compatible  
(C) OPERATING SYSTEM: PC-DOS/MS-DOS  
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

## (vi) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER:  
(B) FILING DATE:  
(C) CLASSIFICATION:

## (viii) ATTORNEY/AGENT INFORMATION:

(A) NAME: ~~Hewitt, Timothy R.~~ *Kowalski, Thomas J.*  
(B) REGISTRATION NUMBER: ~~39,228~~ *32,147*  
(C) REFERENCE/DOCKET NUMBER: ~~TH015~~ *454313-2511.1*

## (ix) TELECOMMUNICATION INFORMATION:

(A) TELEPHONE: ~~(570) 586-1022~~ *212-588-0800*  
(B) TELEFAX: ~~(570) 895-2782~~ *212-588-0500*

## (2) INFORMATION FOR SEQ ID NO:1:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 3701 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

AAGCTTCTAT CAAAAGTCTT AATGAGTTAG GTGTAGATAG TATAGATATT ACTACAAAGG  
60

TATTCATATT TCCTATCAAT TCTAAAGTAG ATGATATTAA TAACTCAAAG ATGATGATAG  
120

TAGATAATAG ATACGCTCAT ATAATGACTG CAAATTGGA CGGTTACAT TTTAATCATC  
180

ACGCGTTCAT AAGTTTCAAC TGCATAGATC AAAATCTCAC TAAAAAGATA GCCGATGTAT  
240

TTGAGAGAGA TTGGACATCT AACTACGCTA AAGAAATTAC AGTTATAAAT AATACATAAT  
300

GGATTTTGTT ATCATCAGTT ATATTTAACA TAAGTACAAT AAAAAGTATT AAATAAAAAT  
360

ACTTACTTAC GAAAAAATGT CATTATTACA AAACTATAT TTTACAGAAC AATCTATAGT  
420

AGAGTCCTTT AAGAGTTATA ATTTAAAAGA TAACCATAAT GTAATATTTA CCACATCAGA  
480

TGATGATACT GTTGTAGTAA TAAATGAAGA TAATGTACTG TTATCTACAA GATTATTATC  
540

ATTTGATAAA ATTCTGTTTT TTAACCTCCTT TAATAACGGT TTATCAAAAT ACGAAACTAT  
600

TAGTGATACA ATATTAGATA TAGATACTCA TAATTATTAT ATACCTAGTT CTTCTTCTTT

660

GTTAGATATT CTAAAAAAA GAGCGTGTGA TTTAGAATTA GAAGATCTAA ATTATGCGTT  
720

AATAGGAGAC AATAGTAACT TATATTATAA AGATATGACT TACATGAATA ATTGGTTATT  
780

TACTAAAGGA TTATTAGATT ACAAGTTTGT ATTATTGCGC GATGTAGATA AATGTTACAA  
840

ACAGTATAAT AAAAAGAATA CTATAATAGA TATAATACAT CGCGATAACA GACAGTATAA  
900

CATATGGGTT AAAAATGTTA TAGAATACTG TTCTCCTGGC TATATATTAT GGTTACATGA  
960

TCTAAAAGCC GCTGCTGAAG ATGATTGGTT AAGATACGAT AACCGTATAA ACGAATTATC  
1020

TGCGGATAAA TTATACACTT TCGAGTTCAT AGTTATATTA GAAAATAATA TAAAACATTT  
1080

ACGAGTAGGT ACAATAATTG TACATCCAAA CAAGATAATA GCTAATGGTA CATCTAATAA  
1140

TATACTTACT GATTTTCTAT CTTACGTAGA AGAACTAATA TATCATCATA ATTCATCTAT  
1200

AATATTGGCC GGATATTTTT TAGAATTCTT TGAGACCACT ATTTTATCAG AATTTATTTT  
1260

TTCATCTTCT GAATGGGTAA TGAATAGTAA CTGTTTAGTA CACCTGAAAA CAGGGTATGA  
1320

AGCTATACTC TTTGATGCTA GTTTATTTTT CCAACTCTCT ACTAAAAGCA ATTATGTAA  
1380

ATATTGGACA AAGAAAACCT TGCAGTATAA GAACTTTTTT AAAGACGGTA AACAGTTAGC  
1440

AAAATATATA ATTAAGAAAG ATAGTCAGGT GATAGATAGA GTATGTTATT TACACGCAGC  
1500

TGTATATAAT CACGTAACCT ACTTAATGGA TACGTTTAAA ATTCTGCTT TTGATTTTAA  
1560

ATTCTCCGGA ATGATAGATA TACTACTGTT TGGAATATTG CATAAGGATA ATGAGAATAT  
1620

ATTTTATCCG AACGCTGTTT CTGTAACTAA TATAATATCA GAATCTATCT ATGCAGATTT  
1680

TTACTTTATA TCAGATGTTA ATAAATTCAG TAAAAAGATA GAATATAAAA CTATGTTTCC  
1740

TATACTCGCA GAAAACTACT ATCCAAAAGG AAGGCCCTAT TTTACACATA CATCTAACGA  
1800

AGATCTTCTG TCTATCTGTT TATGCGAAGT AACAGTTTGT AAAGATATAA AAAATCCATT  
1860

ATTATATTCT AAAAAGGATA TATCAGCAA ACGATTCATA GGTTTATTTA CATCTGTCTG  
1920

TATAAATACG GCTGTTGAGT TAAGAGGATA TAAATAAGA GTAATAGGAT GTTTAGAATG  
1980

GCCTGAAAAG ATAAAAATAT TTAATTCTAA TCCTACATAC ATTAGATTAT TACTAACAGA  
2040

AAGACGTTTA GATATTCTAC ATTCTATCT GCTTAAATTT AATATAACAG AGGATATAGC  
2100

TACCAGAGAT GGAGTCAGAA ATAATTTACC TATAATTTCT TTTATCGTCA GTTATTGTAG  
2160

ATCGTATACT TATAAATTAC TAAATTGCCA TATGTACAAT TCGTGTAAGA TAACAAAGTG  
2220

TAAATATAAT CAGGTAATAT ATAATCCTAT ATAGGAGTAT ATATAATTGA AAAAGTAAAA  
2280

TATAAATCAT ATAATAATGA AACGAAATAT CAGTAATAGA CAGGAACTGG CAGATTCTTC  
2340

TTCTAATGAA GTAAGTACTG CTAAATCTCC AAAATTAGAT AAAAATGATA CAGCAAATAC  
2400

AGCTTCATTC AACGAATTAC CTTTAAATTT TTTCAGACAC ACCTTATTAC AAACATACTA  
2460

AGTCAGATGA TGAGAAAGTA AATATAAATT TAACTTATGG GTATAATATA ATAAAGATTC  
2520

ATGATATTAA TAATTTACTT AACGATGTTA ATAGACTTAT TCCATCAACC CCTTCAAACC  
2580

TTTCTGGATA TTATAAAATA CCACTTAATG ATATTAAAAAT AGATTGTTTA AGAGATGTAA  
2640

ATAATTATTT GGAGGTAAAG GATATAAAAT TAGTCTATCT TTCACATGGA AATGAATTAC  
2700

CTAATATTAA TAATTATGAT AGGAATTTTT TAGGATTAC AGCTGTTATA TGTATCAACA  
2760

ATACAGGCAG ATCTATGGTT ATGGTAAAAC ACTGTAACGG GAAGCAGCAT TCTATGGTAA  
2820

CTGGCCTATG TTTAATAGCC AGATCATTTT ACTCTATAAA CATTTTACCA CAAATAATAG  
2880

GATCCTCTAG ATATTTAATA TTATATCTAA CAACAACAAA AAAATTTAAC GATGTATGGC  
2940

CAGAAGTATT TTCTACTAAT AAAGATAAAG ATAGTCTATC TTATCTACAA GATATGAAAG  
3000

AAGATAATCA TTTAGTAGTA GCTACTAATA TGGAAAGAAA TGTATACAAA AACGTGGAAG  
3060

CTTTTATATT AAATAGCATA TTACTAGAAG ATTTAAAATC TAGACTTAGT ATAACAAAAC  
3120

AGTTAAATGC CAATATCGAT TCTATATTTT ATCATAACAG TAGTACATTA ATCAGTGATA  
3180

TACTGAAACG ATCTACAGAC TCAACTATGC AAGGAATAAG CAATATGCCA ATTATGTCTA

3240

ATATTTTAAAC TTTAGAACTA AAACGTTCTA CCAATACTAA AAATAGGATA CGTGATAGGC  
3300

TGTTAAAAGC TGCAATAAAT AGTAAGGATG TAGAAGAAAT ACTTTGTTCT ATACCTTCGG  
3360

AGGAAAGAAC TTTAGAACAA CTTAAGTTTA ATCAAACCTG TATTTATGAA CACTATAAAA  
3420

AAATTATGGA AGATACAAGT AAAAGAATGG ATGTTGAATG TCGTAGTTTA GAACATAACT  
3480

ATACGGCTAA CTTATATAAA GTGTACGGAC AAAACGAATA TATGATTACT TATATACTAG  
3540

CTCTCATAAG TAGGATTAAT AATATTATAG AAACTTTAAA ATATAATCTG GTGGGGCTAG  
3600

ACGAATCTAC AATACGTAAT ATAAATTATA TAATTTTACA AAGAACAAAA AAAAATCAAG  
3660

TTTCTAATAC CTTATAGATA AACTATATTT TTTACCACTG A  
3701

(2) INFORMATION FOR SEQ ID NO:2:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 42 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

ATCATCGAGC TCGCGGCCGC CTATCAAAAG TCCTAATGAG TT  
42

(2) INFORMATION FOR SEQ ID NO:3:



- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 73 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

GAATTCCTCG AGCTGCAGCC CGGGTTTTTA TAGCTAATTA GTCATTTTTT CGTAAGTAAG  
60

TATTTTTATT TAA  
73

(2) INFORMATION FOR SEQ ID NO:4:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 72 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

CCCCGGCTGC AGCTCGAGGA ATTCTTTTTA TTGATTA ACT AGTCAAATGA GTATATATAA  
60

TTGAAAAAGT AA  
72

(2) INFORMATION FOR SEQ ID NO:5:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 45 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

GATGATGGTA CCTTCATAAA TACAAGTTTG ATTAACTTA AGTTG  
45

(2) INFORMATION FOR SEQ ID NO:6:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 53 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

CATCATCATG ATATCCGTTA AGTTTGTATC GTAATGACGT ATCCAAGGAG GCG  
53

(2) INFORMATION FOR SEQ ID NO:7:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 36 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

TACTACTACG TCGACTTAGG GTTTAAGTGG GGGGTC  
36

(2) INFORMATION FOR SEQ ID NO:8:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 2520 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

GGTACCTTCA TAAATACAAG TTTGATTAAA CTTAAGTTGT TCTAAAGTTC TTTCTCCGA  
60

AGGTATAGAA CAAAGTATTT CTTCTACATC CTTACTATTT ATTGCAGCTT TTAACAGCCT  
120

ATCACGTATC CTATTTTTAG TATTGGTAGA ACGTTTTAGT TCTAAAGTTA AAATATTAGA  
180

CATAATTGGC ATATTGCTTA TTCCTTGCAT AGTTGAGTCT GTAGATCGTT TCAGTATATC  
240

ACTGATTAAT GTACTACTGT TATGATGAAA TATAGAATCG ATATTGGCAT TTAAGTGT  
300

TGTTATACTA AGTCTAGATT TTAAATCTTC TAGTAATATG CTATTTAATA TAAAGCTTC  
360

CACGTTTTTG TATACATTC TTTCCATATT AGTAGCTACT ACTAAATGAT TATCTTCTTT  
420

CATATCTTGT AGATAAGATA GACTATCTTT ATCTTTATTA GTAGAAAATA CTTCTGGCCA  
480

TACATCGTTA AATTTTTTTG TTGTTGTTAG ATATAATATT AAATATCTAG AGGATCCTAT  
540

TATTTGTGGT AAAATGTTTA TAGAGTAAAA TGATCTGGCT ATTAAACATA GGCCAGTTAC  
600

CATAGAATGC TGCTTCCCGT TACAGTGTTT TACCATAACC ATAGATCTGC CTGTATTGTT  
660

GATACATATA ACAGCTGTAA ATCCTAAAAA ATTCCTATCA TAATTATTAA TATTAGGTAA  
720

TTCATTTCCA TGTGAAAGAT AGACTAATTT TATATCCTTT ACCTCCAAAT AATTATTTAC  
780

ATCTCTTAAA CAATCTATTT TAATATCATT AACTGGTATT TTATAATATC CAGAAAGGTT  
840

TGAAGGGGTT GATGGAATAA GTCTATTAAC ATCGTTAAGT AAATTATTAA TATCATGAAT  
900

CTTTATTATA TTATACCCAT AAGTTAAATT TATATTTACT TTCTCATCAT CTGACTTAGT  
960

TAGTTTGTA TAAAGGTGTG CTGAAAAAAT TAAAAGGTAA TTCGTTGAAT GAAGCTGTAT  
1020

TTGCTGTATC ATTTTATCT AATTTTGGAG ATTTAGCAGT ACTTACTTCA TTAGAAGAAG  
1080

AACTGCCAG TTCCTGTCTA TTACTGATAT TTCGTTTCAT TATTATATGA TTTATATTTT  
1140

ACTTTTTCAA TTATATATAC TCATTTGACT AGTTAATCAA TAAAAAGAAT TCCTGCAGCC  
1200

CTGCAGCTAA TTAATTAAGC TACAAATAGT TTCGTTTTCA CCTTGTCTAA TAACTAATTA  
1260

ATTAAGGATC CCCAGCTTC TTTATTCTAT ACTTAAAAAG TGAAAATAAA TACAAAGGTT  
1320

CTTGAGGGTT GTGTAAATT GAAAGCGAGA AATAATCATA AATTATTTCA TTATCGCGAT  
1380

ATCCGTAAAG TTTGTATCGT AATGACGTAT CCAAGGAGGC GTTACCGCAG AAGAAGACAC  
1440

CGCCCCCGCA GCCATCTTGG CCAGATCCTC CGCCGCCGCC CCTGGCTCGT CCACCCCCGC  
1500

CACCGCTACC GTTGGAGAAG GAAAAATGGC ATCTTCAACA CCGCCTCTC CCGCACCTTC  
1560

GGATATACTG TCAAGCGTAC CACAGTCACA ACGCCCTCCT GGGCGGTGGA CATGATGAGA  
1620

TTTAAAATTG ACGACTTTGT TCCCCCGGGA GGGGGGACCA ACAAATCTC TATACCCTTT  
1680

GAATACTACA GAATAAGAAA GGTAAAGGTT GAATTCTGGC CCTGCTCCCC CATCACCAG  
1740

GGTGATAGGG GAGTGGGCTC CACTGCTGTT ATTCTAGATG ATAACCTTTGT AACAAAGGCC  
1800

ACAGCCCTAA CCTATGACCC ATATGTAAAC TACTCCTCCC GCCATACAAT CCCCCAACCC  
1860

TTCTCCTACC ACTCCCGTTA CTTACACCCC AAACCTGTTT TTAGCTCCAC TATTGATTAC  
1920

TTCCAACCAA ATAACAAAAG GAATCAGCTT TGGCTGAGAC TACAAACCTC TGGAAATGTG  
1980

GACCACGTAG GCCTCGGCGC TGCCTTCGAA AACAGTAAAT ACGACCAGGA CTACAATATC  
2040

CGTGTAACCA TGTATGTACA ATTCAGAGAA TTTAATCTTA AAGACCCCCC ACTTAAACCC  
2100

TAAGTCGACC CCGGGTTTTT ATAGCTAATT AGTCATTTTT TCGTAAGTAA GTATTTTTAT  
2160

TTAATACTTT TTATTGTACT TATGTAAAT ATAACCTGATG ATAACAAAAT CCATTATGTA  
2220

TTATTTATAA CTGTAATTTT TTTAGCGTAG TTAGATGTCC AATCTCTCTC AAATACATCG  
2280

GCTATCTTTT TAGTGAGATT TTGATCTATG CAGTTGAAAC TTATGAACGC GTGATGATTA  
2340

AAATGTGAAC CGTCCAAATT TGCAGTCATT ATATGAGCGT ATCTATTATC TACTATCATC  
2400

ATCTTTGAGT TATTAATATC ATCTACTTTA GAATTGATAG GAAATATGAA TACCTTTGTA  
2460

GTAATATCTA TACTATCTAC ACCTAACTCA TTAAGACTTT TGATAGGCGG CCGCGAGCTC

2520

(2) INFORMATION FOR SEQ ID NO:9:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 57 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

CATCATCATT CGCGATATCC GTTAAGTTTG TATCGTAATG CCCAGCAAGA AGAATGG  
57

(2) INFORMATION FOR SEQ ID NO:10:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 36 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

TACTACTACG TCGACTCAGT AATTTATTTTC ATATGG  
36

(2) INFORMATION FOR SEQ ID NO:11:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 3609 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

GGTACCTTCA TAAATACAAG TTTGATTAAA CTTAAGTTGT TCTAAAGTTC TTTCTCCGA

60

AGGTATAGAA CAAAGTATTT CTTCTACATC CTTACTATTT ATTGCAGCTT TTAACAGCCT

120

ATCACGTATC CTATTTTTAG TATTGGTAGA ACGTTTTAGT TCTAAAGTTA AAATATTAGA

180

CATAATTGGC ATATTGCTTA TTCCTTGCAT AGTTGAGTCT GTAGATCGTT TCAGTATATC

240

ACTGATTAAT GTACTACTGT TATGATGAAA TATAGAATCG ATATTGGCAT TTAAGTGT

300

TGTTATACTA AGTCTAGATT TTAAATCTTC TAGTAATATG CTATTTAATA TAAAAGCTTC

360

CACGTTTTTG TATACATTTT TTTCCATATT AGTAGCTACT ACTAAATGAT TATCTTCTTT

420

CATATCTTGT AGATAAGATA GACTATCTTT ATCTTTATTA GTAGAAAATA CTTCTGGCCA

480

TACATCGTTA AATTTTTTTG TTGTTGTTAG ATATAATATT AAATATCTAG AGGATCCTAT

540

TATTTGTGGT AAAATGTTTA TAGAGTAAAA TGATCTGGCT ATTAAACATA GGCCAGTTAC

600

CATAGAATGC TGCTTCCCGT TACAGTGTTC TACCATAACC ATAGATCTGC CTGTATTGTT

660

GATACATATA ACAGCTGTAA ATCCTAAAAA ATTCCTATCA TAATTATTAA TATTAGGTAA

720

TTCATTTCCA TGTGAAAGAT AGACTAATTT TATATCCTTT ACCTCCAAAT AATTATTTAC

780

ATCTCTTAAA CAATCTATTT TAATATCATT AACTGGTATT TTATAATATC CAGAAAGGTT

840

TGAAGGGGTT GATGGAATAA GTCTATTAAC ATCGTTAAGT AAATTATTAA TATCATGAAT  
900

CTTTATTATA TTATACCCAT AAGTTAAATT TATATTTACT TTCTCATCAT CTGACTTAGT  
960

TAGTTTGTA TAAAGGTGTGT CTGAAAAAAT TAAAAGGTAA TTCGTTGAAT GAAGCTGTAT  
1020

TTGCTGTATC ATTTTTATCT AATTTTGGAG ATTTAGCAGT ACTTACTTCA TTAGAAGAAG  
1080

AATCTGCCAG TTCCTGTCTA TTA CTGATAT TTCGTTTCAT TATTATATGA TTTATATTTT  
1140

ACTTTTTCAA TTATATATAC TCATTTGACT AGTTAATCAA TAAAAAGAAT TTCGACTTAG  
1200

GGTTTAAGTG GGGGGTCTTT AAGATTAAAT TCTCTGAATT GTACATACAT GGTTACACGG  
1260

ATATTGTAGT CCTGGTCGTA TTTACTGTTT TCGAACGCAG CGCCGAGGCC TACGTGGTCC  
1320

ACATTTCCAG AGGTTTGTAG TCTCAGCCAA AGCTGATTCC TTTTGTTATT TGGTTGGAAG  
1380

TAATCAATAG TGGAGTCAAG AACAGGTTTG GGTGTGAAGT AACGGGAGTG GTAGGAGAAG  
1440

GGTTGGGGGA TTGTATGGCG GGAGGAGTAG TTTACATATG GGTCATAGGT TAGGGCTGTG  
1500

GCCTTTGTGA CAAAGTTATC ATCTAGAATA ACAGCAGTGG AGCCCACTCC CCTATCACCC  
1560

TGGGTGATGG GGGAGCAGGG CCAGAATTCA ACCTTAACCT TTCTTATTCT GTAGTATTCA  
1620

AAGGGTATAG AGATTTTGTG GGTCCCCCT CCCGGGGGAA CAAAGTCGTC AATTTTAAAT  
1680



CTCATCATGT CCACCGCCCA GGAGGGCGTT GTGACTGTGG TACGCTTGAC AGTATATCCG  
1740

AAGGTGCGGG AGAGGCGGGT GTTGAAGATG CCATTTTTC TTCTCCAACG GTAGCGGTGG  
1800

CGGGGGTGGA CGAGCCAGGG GCGGCGGCGG AGGATCTGGC CAAGATGGCT GCGGGGGCGG  
1860

TGTCTTCTTC TGCGGTAACG CCTCCTTGGA TACGTCATTA CGATACAAAC TTAACGGATA  
1920

TCGCGATAAT GAAATAATTT ATGATTATTT CTCGCTTTCA ATTTAACACA ACCCTCAAGA  
1980

ACCTTTGTAT TTATTTTTCAC TTTTAAAGTA TAGAATAAAG AAGCTGGGGG ATCAATTCCT  
2040

GCAGCCCTGC AGCTAATTAA TTAAGCTACA AATAGTTTCG TTTTCACCTT GTCTAATAAC  
2100

TAATTAATTA AGGATCCCCC AGCTTCTTTA TTCTATACTT AAAAAGTGAA AATAAATACA  
2160

AAGGTTCCTG AGGGTTGTGT TAAATTGAAA GCGAGAAATA ATCATAAATT ATTCATTAT  
2220

CGCGATATCC GTTAAGTTTG TATCGTAATG CCCAGCAAGA AGAATGGAAG AAGCGGACCC  
2280

CAACCACATA AAAGGTGGGT GTTCACGCTG AATAATCCTT CCGAAGACGA GCGCAAGAAA  
2340

ATACGGGAGC TCCCAATCTC CCTATTGAT TATTTTATTG TTGGCGAGGA GGGTAATGAG  
2400

GAAGGACGAA CACCTCACCT CCAGGGGTTG GCTAATTTTG TGAAGAAGCA AACTTTTAAT  
2460

AAAGTGAAGT GGTATTTGGG TGCCCGCTGC CACATCGAGA AAGCCAAAGG AACTGATCAG  
2520

CAGAATAAAG AATATTGCAG TAAAGAAGGC AACTTACTTA TTGAATGTGG AGCTCCTCGA

PAGE MISSING AT TIME OF PUBLICATION

TGATGATTAA AATGTGAACC GTCCAAATTT GCAGTCATTA TATGAGCGTA TCTATTATCT  
3480

ACTATCATCA TCTTTGAGTT ATTAATATCA TCTACTTTAG AATTGATAGG AAATATGAAT  
3540

ACCTTTGTAG TAATATCTAT ACTATCTACA CCTAACTCAT TAAGACTTTT GATAGGCGGC  
3600

CGCGAGCTC  
3609

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 53 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

CATCATCATG ATATCCGTTA AGTTTGTATC GTAATGACGT GGCCAAGGAG GCG  
53

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 40 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

WO 00/77216

PCT/IB00/00882

TACTACTACG TCGACTTATT TATTTAGAGG GTCTTTTAGG

40